nature

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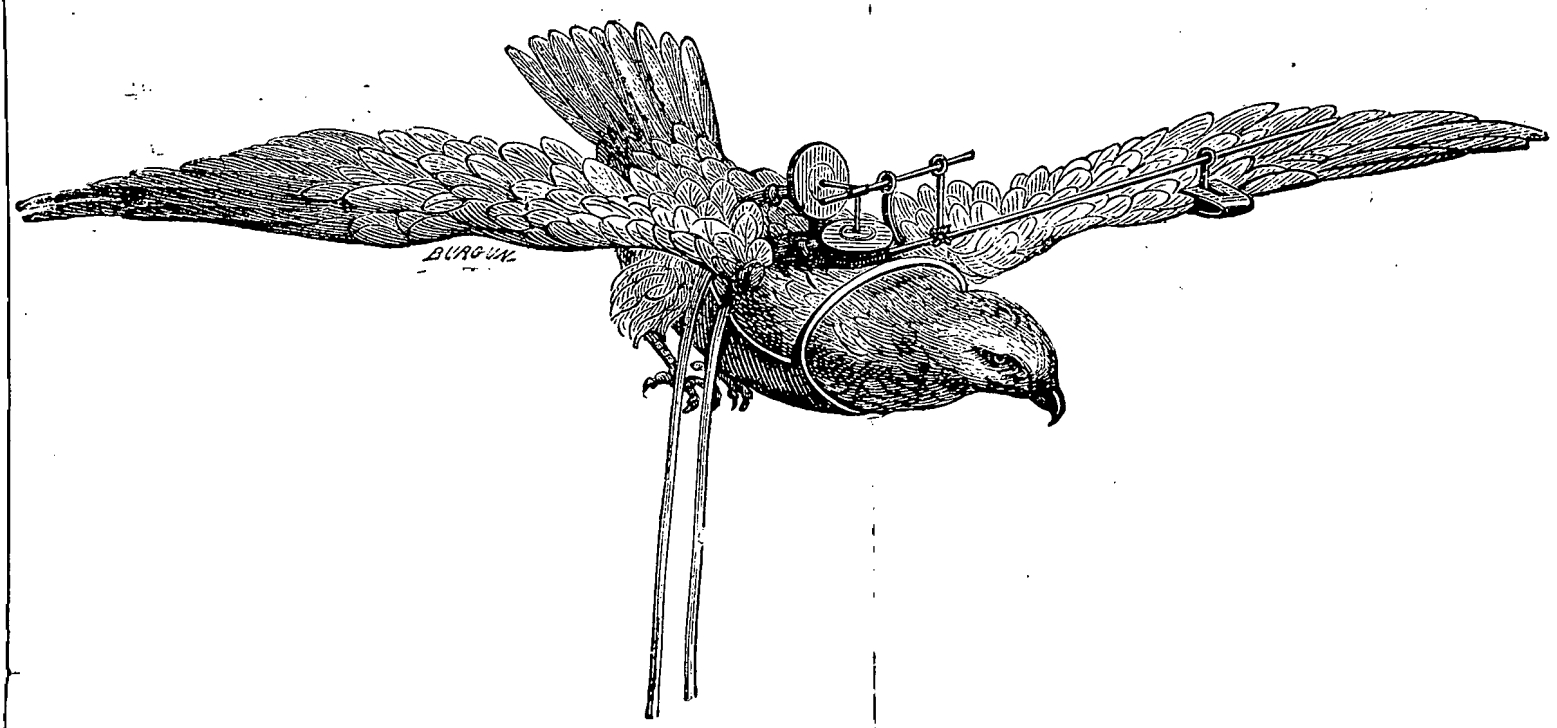
almost astonishment to all readers of his work.

Fig. 5 shows a buzzard saddled with the machinery which, by means of the two tubes running downwards from it, transmits the vertical and horizontal movements of its wing to the recording apparatus, which is not represented. In the study of the more intricate points the



necessary instruments are so heavy that the whole bird has to be partially supported. This is done by attaching it to the extremity of a long lever which revolves, with scarcely any friction, on a pivot. This is found not seriously to interfere with the normal flight of the bird.

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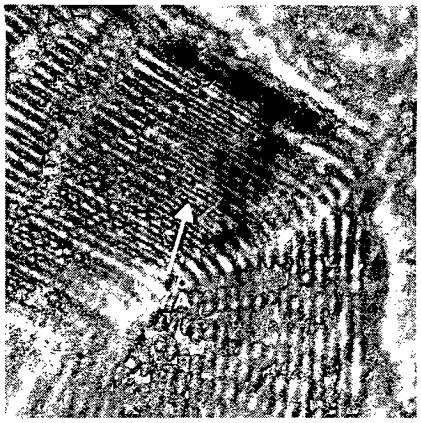

Above left
Graphitised carbon lattice showing 1,7Å separation
Above centre
(220) lattice fringes of Diamond, 1,2Å separation
Above right
Liver DNA and Cytochrome C showing spiral structure 100Å

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PHILIPS

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Cover Picture

A hundred years ago *Nature* was reviewing E. J. Marey's *Animal Mechanism* (page 518, October 29, 1874). These cumbersome mechanisms were soon to be replaced by Muybridge's zoopraxiscope camera. On page 567 we look at a Muybridge sequence and—a century later—what happens when the light is switched on.

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Guide to authors

Nature accepts three types of communications:

● Articles are up to 3,000 words in length with at most six displayed items (figures and tables) and may either be reports of major research developments in a subject or broader reviews of progress.

● Letters are brief reports of research of unusual and wide interest, not in general longer than 1,000 words; at most they have three or four displayed items (figures and tables).

● 'Matters Arising' permits occasional short discussion of papers that have previously appeared in *Nature*. A limit of 300 words is placed on contributions in this category.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the Système International. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible. exp (*a*) is preferred to e^a if '*a*' is more than one character. Articles should be accompanied by an abstract of not more than fifty words, and the abstract should list the main conclusions that are drawn.

References are indicated by superscripts in the text. The style may be gleaned from any contemporary *Nature* with the following two changes:

(i) If it is necessary to refer to several references by the same author at once, only one reference number need be given.

(ii) The last page as well as the first of any reference should be cited.

Abbreviations should follow the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). Symposia are often difficult to refer to and only published or soon-to-be-published volumes should be mentioned in references. Their publisher and place of publication should be clearly indicated. 'Personal communication' and 'unpublished work' should be incorporated in the text.

Artwork should be sent with the manuscript. All artwork should be marked with the author's name. Line drawings should preferably be in Indian ink on heavy cartridge paper, although other materials are acceptable; thin, shiny, folded, torn or heavily handled material should be avoided. Matt rather than glossy photographs are preferred. Figures are usually reduced to one column width. The originals should be about as wide as a page of *Nature*. Figures, particularly maps, should contain nothing but essential material. It is preferred that the original be unlabelled, but with a copy containing lettering. Labelling on photographs should if possible be avoided entirely.

A fuller guide appeared in *Nature* (**246**, 238; 1973).

nature

Volume 251 October 18, 1974

Investigating the paranormal

WE publish this week a paper by Drs R. Targ and H. Puthoff (page 602) which is bound to create something of a stir in the scientific community. The claim is made that information can be transferred by some channel whose characteristics appear to fall "outside the range of known perceptual modalities". Or, more bluntly, some people can read thoughts or see things remotely.

Such a claim is, of course, bound to be greeted with a preconditioned reaction amongst many scientists. To some it simply confirms what they have always known or believed. To others it is beyond the laws of science and therefore necessarily unacceptable. But to a few—though perhaps to more than is realised—the questions are still unanswered, and any evidence of high quality is worth a critical examination.

The issue, then, is whether the evidence is of sufficient quality to be taken seriously. In trying to answer this, we have been fortunate in having the help of three independent referees who have done their utmost to see the paper as a potentially important scientific communication and not as a challenge to or confirmation of prejudices. We thank them for the considerable effort they have put in to helping us, and we also thank Dr Christopher Evans of the National Physical Laboratory whose continued advice on the subject is reflected in the content of this leading article.

A general indication of the referees' comments may be helpful to readers in reaching their own assessment of the paper. Of the three, one believed we should not publish, one did not feel strongly either way and the third was guardedly in favour of publication. We first summarise the arguments against the paper.

(1) There was agreement that the paper was weak in design and presentation, to the extent that details given as to the precise way in which the experiment was carried out were disconcertingly vague. The referees felt that insufficient account had been taken of the established methodology of experimental psychology and that in the form originally submitted the paper would be unlikely to be accepted for publication in a psychological journal on these grounds alone. Two referees also felt that the authors had not taken into account the lessons learnt in the past by parapsychologists researching this tricky and complicated area.

(2) The three referees were particularly critical of the method of target selection used, pointing out that the choice of a target by "opening a dictionary at random" is a naive, vague and unnecessarily controversial approach to randomisation. Parapsychologists have long rejected such methods of target selection and, as one referee put it, weaknesses of this kind reveal "a lack of skill in their experiments, which might have caused them to make some other mistake which is less evident from their writing".

(3) All the referees felt that the details given of various safeguards and precautions introduced against the possibility of conscious or unconscious fraud on the part of one or other of the subjects were "uncomfortably vague" (to use one phrase). This in itself might be sufficient to raise doubt that the experiments have demonstrated the existence of a new channel of communication which does not involve the use of the senses.

(4) Two of the referees felt that it was a pity that the paper, instead of concentrating in detail and with meticulous care on one particular approach to extra-sensory phenomena, produced a mixture of different experiments, using different subjects in unconnected circumstances and with only a tenuous overall theme. At the best these were more "a series of pilot studies . . . than a report of a completed experiment".

On their own these highly critical comments could be grounds for rejection of the paper, but it was felt that other points needed to be taken into account before a final decision could be made.

(1) Despite its shortcomings, the paper is presented as a scientific document by two qualified scientists, writing from a major research establishment apparently with the unqualified backing of the research institute itself.

(2) The authors have clearly attempted to investigate under laboratory conditions phenomena which, while highly implausible to many scientists, would nevertheless seem to be worthy of investigation even if, in the final analysis, negative findings are revealed. If scientists dispute and debate the reality of extra-sensory perception, then the subject is clearly a matter for scientific study and reportage.

(3) Very considerable advance publicity—it is fair to say not generated by the authors or their institute—has preceded the presentation of this report. As a result many scientists and very large numbers of non-scientists believe, as the result of anecdote and hearsay, that the Stanford Research Institute (SRI) was engaged in a major research programme into parapsychological matters and had even been the scene of a remarkable breakthrough in this field. The publication of this paper, with its muted claims, suggestions of a limited research programme, and modest data, is, we believe, likely to put the whole matter in more reasonable perspective.

(4) The claims that have been made by, or on behalf of, one of the subjects, Mr Uri Geller, have been hailed publicly as indicating total acceptance by the SRI of allegedly sensational powers and may also perhaps now be seen in true perspective. It must be a matter of interest to scientists to note that, contrary to very widespread rumour, the paper does not present any evidence whatsoever for Geller's alleged abilities to bend metal rods by stroking them, influence magnets at a distance, make watches stop or start by some psychokinetic force and so on. The publication of the paper would be justified on the grounds of allowing scientists the opportunity to discriminate between the cautious, limited and still highly debatable experimental data, and extravagant rumour, fed in recent days by inaccurate attempts in some newspapers at precognition of the contents of the paper.

(5) Two of the referees also felt that the paper should be published because it would allow parapsychologists, and all other scientists interested in researching this arguable field, to gauge the quality of the Stanford research and assess how much it is contributing to parapsychology.

(6) *Nature*, although seen by some as one of the world's most respected journals cannot afford to live on respectability. We believe that our readers expect us to be a home for the occasional 'high-risk' type of paper. This is hardly to assert that we regularly fly in the face of referees' recommendations (we always consider the possibility of publishing, as in this case, a summary of their objections). It is to say that the unusual must now and then be allowed a toe-hold in the literature, sometimes to flourish, more often to be forgotten within a year or two.

The critical comments above were sent to the authors who have modified their manuscript in response to them. We have also corresponded informally with the authors on one or two issues such as whether the targets could have been forced by standard magical tricks, and are convinced that this is not the case. As a result of these exchanges and the above considerations we have decided to publish in the belief that, however flawed the experimental procedure and however difficult the process of distilling the essence of a complex series of events into a scientific manuscript, it was on balance preferable to publish and maybe stimulate and advance the controversy rather than keep it out of circulation for a further period.

Publishing in a scientific journal is not a process of receiving a seal of approval from the establishment; rather it is the serving of notice on the community that there is something worthy of their attention and scrutiny. And this scrutiny is bound to take the form of a desire amongst some to repeat the experiments with even more caution. To this end the *New Scientist* does a service by publishing this week the results of Dr Joe Hanlon's own investigations into a wide range of phenomena surrounding Mr Geller. If the subject is to be investigated further—and no scientist is likely to accept more than that the SRI experiments provide a *prima facie* case for more investigations—the experimental technique will have to take account of Dr Hanlon's strictures, those of our own referees and those, doubtless, of others who will be looking for alternative explanations.

Perhaps the most important issue raised by the circumstances surrounding the publication of this paper is whether science has yet developed the competence to confront claims of the paranormal. Supposedly paranormal events frequently cannot be investigated in the calm, controlled and meticulous way that scientists are expected to work, and so there is always a danger that the investigator, swept up in the confusion that surrounds many experiments, abandons his initial intentions in order to go along with his subject's desires. It may be that all experiments of this sort should be exactly prescribed beforehand by one group, done by another unassociated group and evaluated in terms of performance by the first group. Only by increasing austerity of approach by scientists will there be any major progress in this field.

For those in peril on the factory floor

In this article Peter J. Smith argues that a greater commitment (in deed as well as word) to community science by the Scientific Establishment might help the world of science regain some of the public respect it has lost.

THE question of who speaks, or should speak, on behalf of the scientific community has been debated on many occasions, most often without result. On the face of it, such lack of resolution is hardly unexpected, for scientists and scientific institutions are not noted for their ready ability to achieve consensus. Yet there is no doubt that they can put up a pretty collective front when they feel so moved. The one famous occasion on which a near consensus was reached was when the scientific community saw itself put at risk financially by the Rothschild proposals. Then individuals and institutions miraculously found a common cause of self-preservation.

But when it comes to the defence of less privileged groups it is quite a different story; the voice of the British scientific community is seldom to be heard, whether taking a moral stance, exerting humanitarian pressure, supplying expertise or even simply providing information. A good case in point is provided by a new *Socialist Worker* pamphlet entitled *Asbestos: The Dust that Kills in the Name of Profit*. As the title hints, the object of *Socialist Worker* is nothing less than the complete overthrow of the capitalist system; and one of the ways of achieving this aim, it seems, is to give strident publicity to defects in the capitalist-industrial system. Fortunately, one can easily avoid a sharp turn to the left and still admit that what some British workers have been subjected to in the name of asbestos production is beyond the limit of acceptability in a humanitarian society.

For what clearly emerges from the rhetoric of the pamphlet in question is a picture of men and women reacting in some bewilderment to the long-term ill effects of a technological activity. The chief consequence is, of course, asbestosis—a killing disease acquired by breathing in asbestos fibres. The bulk of the pamphlet is devoted to case histories of men to whom asbestosis has come as a shock after a decade or so in the industry. But more instructively, there is also a short account of the fight for safety put up by a small group of the 7/162 Glasgow insulation workers' branch of the Transport and General Workers Union against the obstruction of the asbestos companies, the indifference of politicians, the weakness of the Factory Inspectorate, the silence of much of the press, the impotence of health authorities, the equivocal official stance of unions in general, and, last but not least, apathy among many of the asbestos workers themselves.

And there is certainly something to fight about. According to Patrick Kinnersly (*The Hazards of Work: How to Fight Them*, Pluto Press, 1973), asbestosis is taking an increasing toll: 64 are known to have died in 1965, 107 in 1970 and 113 in 1971. The number of new cases diagnosed rose from 82 in 1965 to 153 in 1970. Moreover, asbestosis is only one of the asbestos-induced diseases. Lung cancer appears to require a smaller exposure to asbestos. There is also another form of cancer known as mesothelioma which involves growths in the linings of the lungs and stomach. Almost all mesotheliomas are caused by asbestos; but no one knows how many workers in Britain are killed by them, partly because they take so long to develop and partly because they are not always identified. The TUC Centenary Institute of Occupational Health has suggested that, 30 years after first exposure, about one in 200 will be found to have died of mesothelioma; but Dr Irving J. Selikoff of Mount Sinai Hospital in New York is apparently more pessimistic. He has recently been quoted as saying that, for every 100,000 workers entering the asbestos industry under the safety standards obtaining in the United States as recently as 1971, he would expect 20,000 to die of lung cancer, 7,000 of mesothelioma and 7,000 of other cancers and asbestosis.

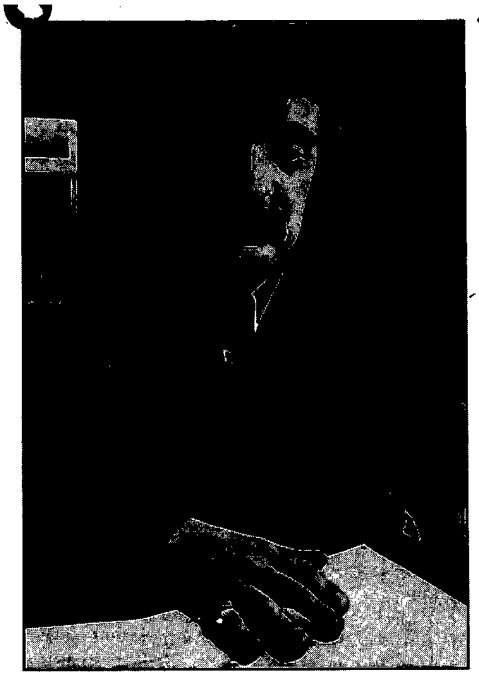
The point of drawing attention to the asbestos problem is not to elevate it into some sort of special case; any one of a hundred examples could just as easily be used for illustration. Nor is it to argue that the whole asbestos industry should be closed down forthwith. Such a course of action would be inconceivable in the face of the fact that asbestos currently finds some 3,000 uses in almost every sphere of material living (a testimony to its unique combination of fibrosity and imperviousness to heat). Asbestos products (and thousands of other equally or more dangerous substances) are here to stay.

But under what conditions are they to stay? And who is to influence those conditions? The most striking thing to emerge from the story of the asbestos workers of Glasgow and elsewhere is that no organisation remotely connected with the world of established science has played any part in it. The British Association has been silent. The Science and Natural Environment Research Councils have been silent. The Royal Institution has been silent. The University Grants Committee has been silent. The Association of University Teachers has been silent. In this and many other comparable cases a few individual scientists and science journalists have spoken and acted; but what has been completely lacking is the evidence of any sense of collective responsibility on behalf of the whole, or even a significant part, of the British scientific community.

It is true of course, that the Medical Research Council and the recipients of their grants are responsible for a great deal of basic biological and medical research into this and other industrial diseases; and my remarks should not be construed as criticism of that organisation, the individuals associated with it or the valuable work they do. The point I make is much more general—that even when the adverse side-effects of science and technology directly involve health (and they do not always do so), their mitigation requires much more than fundamental research into the nature of the disease itself.

Here, with particular reference to the asbestos case but with general applicability to many other situations, are some specific things which the scientific community could (and should) have done and could still do.

- Admit moral responsibility for taking a clear lead in seeing that the ill effects of science and technology are eliminated or at least mitigated. As Dr. Bernard Dixon (*What is Science for?*, Collins, 1973) has said: "When an unexpected, unforeseen calamity does happen, the scientist is more, not less or equally, responsible compared with others in doing all he can to fight against it". In practical terms, taking a moral lead would probably first mean overseeing the organisation of a suitable team to map out the full extent of the problem and identify gaps in existing knowledge. Between them, the organisations mentioned above encompass the elite (albeit often self-elected), if not most participants, of British science, and form a body with an almost infinite variety of expertise and an immense potential for influencing public and government opinion. That these powers are so often allowed to lie dormant is, to say the least, astonishing.
- Ensure that the outstanding questions and problems identified by the investigating team are followed through. In almost all cases it is probable that more research is needed, in which case the distinguished bodies of science should regard it as their duty to create the necessary political, financial and organisational climate in which such research can be carried out. Is monitoring equipment highly enough developed? Are safety levels adequate and are they formulated in the right way? To what extent is the general public, as well as factory workers, at risk? These and many other questions covering a variety of disciplines can be investigated but they require effort. Again, the principal scientific organisations have it in their power to encourage the mobilisation of scientists for this purpose. Will they use this influence?
- Exert pressure to ensure that the high principles so proudly proclaimed by the scientific community are actually put into effect. For example, it is quite plain that one of the greatest difficulties encountered by industrial workers and others exposed to risk is that of securing the scientific data (for example, from monitoring) from which a proper assessment risk may be made. Up to July of this year, Factory Inspectors divulging such information to employees or others without the employer's permission were actually subject to imprisonment. The Health and Safety at Work Act 1974 has mitigated these harsh conditions, although as the Trade Union Research Unit at Oxford has pointed out (*Health and Safety at Work Legislation: A Critical Study*, 1974), disclosure of information is still left largely to the Factory Inspector's discretion. It is difficult to understand how a community which puts such a high value on the freedom of scientific information can reasonably continue to allow the principle to be treated with contempt. Is there any respectable reason why human health should be less privileged in this respect than the activity of pure science? Of course, employers frequently attempt to justify their refusal to disclose vital information on the grounds of commercial secrecy—a ploy which is sometimes legitimate, sometimes not. The scientific community has a unique role to play here, for a group of independent scientific experts of integrity is the only body able to make a proper assessment of what is truly confidential and what is unreasonably claimed as such.



A worker displays symptoms of asbestosis

Why has the scientific community as a whole failed in this respect? The reason seems to be that, in the past, scientists have found it adequate to justify widespread public support of science on the grounds of the supposed cultural value of the activity and of its supposed role as the progenitor of technology. But what they have failed to grasp is that the first of these grounds was never very convincing to the wider world (at least as justification for a high level of public support) and that the second is rapidly losing its credibility.

In fact, science has a third role, which is neither "science for its own sake" nor science conceived as a means to a materialistic end. This is science for human welfare—call it "community science" if you like. It is practised in a few university departments; it is practised with great effect by, for example, the staff of the Greater London Council's Scientific Adviser. It is regarded as second rate by most university scientists, as a threat by industry, and with indifference by our major scientific institutions. But it is just possible that a greater commitment to it by the scientific community might go some way towards regaining for science the public respect (not to mention the public money) it has so patently lost in recent years. □

international news

Frameworks for energy in the US...

by Colin Norman, Washington

ON THE eve of its recess for the November elections, the US Congress approved what could turn out to be the most significant piece of energy-related legislation to reach the lawbooks since the oil crisis began to bite.

Minutes before President Ford appeared before a joint session of Congress to deliver his economic pep talk, a House-Senate Conference committee reached agreement on a bill which will abolish the Atomic Energy Commission and replace it with a Nuclear Regulatory Commission (NRC) and an independent Energy Research and Development Administration (ERDA). Two days later, the bill was sitting on Ford's desk awaiting his signature. Until last week, the bill had been making glacial passage through the Congressional mill, and there were fears that no agreement would be reached on the measure before the end of the session.

The importance of the legislation is that it consolidates energy research and development programmes which are now spread out over a host of federal departments and agencies into a single agency. Furthermore, it removes a long standing complaint that the Atomic Energy Commission suffers from a conflict of interest by both promoting and regulating nuclear energy, because it splits those functions into separate agencies. Furthermore, the bill considerably elevates the status of research programmes concerned with nuclear safety and the safeguarding of nuclear materials.

The bill, which was originally proposed by former President Nixon's Administration, sets up the NRC to take over virtually all of the regulatory functions that are now performed by the Atomic Energy Commission, but it also completely alters the present bureaucratic structure. The NRC will consist of three co-equal offices, each of which will report to a 5-member commission. The Office of Nuclear Reactor Regulation will be concerned with licensing nuclear reactors. The Office of Material Safety and Safeguards will license reprocessing facilities and facilities associated with transporting nuclear materials, and it will also make sure that nuclear materials are adequately safeguarded.

The third, the Office of Nuclear Regulatory Research, will perform backup research for evaluating licence applications to ensure that safety and safeguards criteria are met.

As for ERDA, it will take over virtually all the energy research and development activities performed by the federal government, using the laboratories of the Atomic Energy Commission as the base of its operations. It will be split into six divisions, each of which will be headed by an Assistant Administrator, concerned with fossil fuels; nuclear energy; environmental safety; conservation, solar, geothermal, and advanced energy systems; and nuclear security.

ERDA will carry out all the energy programmes related to Project Independence—the much vaunted drive to make the United States self-sufficient in energy supplies—and it will be armed with a huge budget, amounting at present to about $2,000 million a year. Nuclear energy will soak up nearly half of the ERDA funds, with the breeder reactor getting the biggest single slice. But it will also take over such programmes as the solar energy research effort which was recently launched by Congress (see *Nature*, 251, 368, 1974), as well as Congressionally inspired research and development programs concerned with geothermal energy and other longer-term energy options. □

... and in Holland

from Arie de Kool, Amsterdam

THE Netherlands are going nuclear, but slowly, and with a maximum of government control. In fact, the government will increase its influence in the whole of the energy field considerably—if possible, together with other European countries, if not, alone.

Its proposals are set out in a long-awaited White Book on Energy. Several of these are concerned with getting the government a measure of control in the privately owned energy supply sector. As far as nuclear power is concerned, the government proposes to establish a monopoly for the exploitation of nuclear power stations and promises to undertake studies on overall nuclear safety within two years. It plans to build three 1,000 MW nuclear power stations, to be on stream by 1985 or when the establishment of the government nuclear monopoly and the safety studies permit.

The government also wants to negotiate a say in the managerial conditions of energy supply corporations, and by bringing the planning of all types of power stations under government responsibility, ensure that electrical power supply is discussed in parliament. To stimulate the use of coal for electricity generation the government will stipulate that all new power plants shall be fitted for oil and coal firing. The Dutch will also continue to participate in the German-Belgian-Dutch prototype sodium-cooled fast breeder reactor at Kalkar in Germany, although the government is strongly considering pulling out of the next phase, the construction of a 1,000 MW demonstration reactor.

Looking outwards, the government plans to take a 40% share in all natural gas and oil exploitations on the Dutch part of the continental shelf, with the condition that the government share in the profit may rise as high as 80% if conditions allow "without impeding the attractiveness to the oil companies". The Dutch government also plans to build up a "strategic reserve" of natural gas by stimulating exploration and exploitation of smaller fields in order to have the major source at Slochteren available in case of another oil crisis.

It is no secret, that the cabinet has been highly divided, especially over the use of nuclear energy. A couple of days before the White Book was officially released, it leaked out that the government proposed a considerable delay in the construction of nuclear power plants. This was the interpretation the 'progressive' members of the cabinet were giving (and were supposed to give) to the delay that is bound to result from the establishment of the government monopoly and the requirements of an overall safety report.

However, Dr Lubbers, Minister of Economic Affairs, maintained at a press conference, that the three plants should be ready by 1985. The safety reports might lead to a change in reactor type, a change of site, even to a postponement of the start of the first reactor, but not to the goal of 3,500 MW of nuclear energy in 1985.

Naturally, the opponents of nuclear energy are not very happy with the White Book. Some 10,000 people biked and bussed to Kalkar in protest, three days after the White Book was published. □

The chemistry of profits

from Nechemia Meyers, Rehovot

DETAILED plans are now being made for an investment of some £240 million in Israel's chemical industry. Roughly half this sum will be used to promote the exploitation of inorganic Negev chemicals, with the other half being used to expand the Haifa Bay complex of petrochemical firms.

The investment, enormous in Israeli terms, is a natural outcome of the chemical industry's growing success, both in supplying vital products to the home market and in the export sphere. Overseas sales of Israeli chemicals already account for 20% of her industrial exports (excluding diamonds), and there is no apparent limit to potential customers.

The 393-square-mile Dead Sea, most saline of the world's lakes, has long been the major focus of Israel's chemical industry in the 20th century. Plans for exploiting its potash and other mineral resources were drawn up in 1911 by Moshe Novomeysky (an immigrant from Siberia) and the first plant, run by Novomeysky, began operation in 1931. For many years, however, the Dead Sea Works were usually in the red, causing cynics to suggest that "the only thing that can sink in the Dead Sea is money".

It sinks no longer. On the contrary, Dead Sea potash, bromine and magnesium oxide—extracted from a 50-square-mile area of evaporation pans at the southern end of the saline lake whose waters initially contain only 1% (by weight) of potassium chloride—are today sold at a handsome profit to customers in Europe, Africa and the Far East.

Other Negev enterprises are also profitable, in large measure because of rising prices. Israel's phosphate mines—part of the Mediterranean phosphate belt that stretches from Morocco in the west to Jordan in the east and Turkey in the north—are a striking case in point.

In 1971 Government experts, dismayed by 20 years of losses, suggested that they be closed down. Fortunately they were not, and as a result profits are now rolling in to the State Treasury. Ironically, they largely reflect Arab Morocoo's successful exploitation of the world-wide fertiliser shortage to push up phosphate prices from $11 to $60 a ton in just one year.

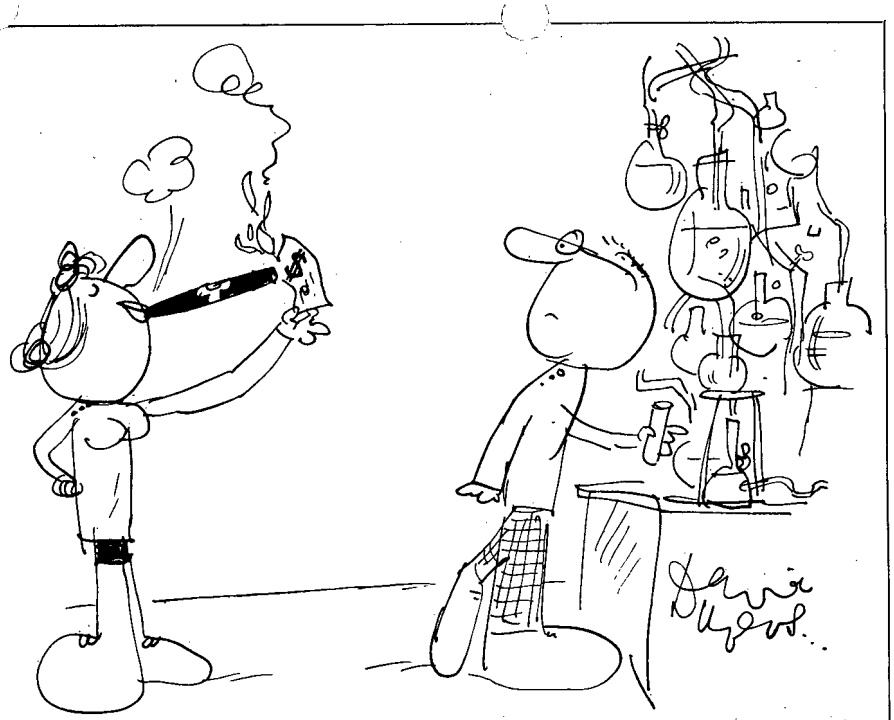
Copper prices are now lower than they were at their peak, but they are still high enough to ensure the profitability of the modern copper mines at Timna (which are very near the Chalcolithic ones). However, the copper-bearing strata that were worked in ancient times are not the same as those mined today. Formerly copper nodules associated with fossilised trees within the Middle White Sandstone were exploited. This layer appears near Timna about 330 feet above the copper-bearing Cambrian rocks that are now worked (by both underground and open-cast mining).

The only major failure in this sphere has been with a controversial plant, near the Judean Desert town of Arad, for the manufacture of phosphoric acid by means of hydrochloric acid produced from residual Dead Sea brines. An American firm whose fluidised refining process was used in the plant, the Madera Company, is being sued in an attempt to recover at least part of the £25 million loss, and alternative operating methods are now being studied.

When the current expansion plan is completed, potash production will rise from one million to 1.5 million tons a year, phosphate production from one million to 2.8 million tons a year and bromine production from 20,000 to 50.000 tons annually. Profits from the mining and processing of Negev chemicals, this year expected to total £15 million, will presumably also rise in at least the same measure.

The scheduled £240 million investment in the petrochemical industry will meet local needs for petrochemicals (including raw materials for plastics) and also give a boost to exports. Items whose production is to rise include polyethylene, styrene, polystyrene, phenolics and PVC. In addition, much larger quantities of potassium nitrate — a highly regarded, Israeli-developed chemical fertilizer—will be produced in the Haifa area.

While the cost of the oil used as a base for these petrochemical products has obviously risen steeply, Israel is no worse off in this respect than her competitors, and looks ahead to profits from petrochemicals in addition to those she is already earning from inorganic chemicals. □



WOMEN who graduated from American universities this year with a degree in chemistry or chemical engineering commanded higher salaries than their male counterparts, according to a study carried out by the American Chemical Society. It is the first time since the society began keeping track of such matters that women graduates have earned more than men.

The ACS survey showed that, on average, the starting salary for women chemists this year was 5% higher than that for men, whereas in 1964, newly graduated women chemists could expect to earn only 68% of the starting salary for men. While this seems to indicate that employers are attempting to recruit more women, the survey also turned up evidence that the battle for equal employment opportunities still has some way to go. For one thing, women and minority groups had a higher unemployment rate than the average for all ACS members. The overall rate of 1.4% was exceeded by American Indians (6.3%), Orientals (3.1%), Spanish surnamed and black chemists (1.5%), and women (3.5%). But at least that's an improvement on previous years, when the unemployment rate among women chemists was typically running at about three times that for men.

ICI puts money on genetic engineering

by Miranda Robertson

THE news that Britain's Imperial Chemical Industries Ltd (ICI) is to invest £40,000 over the next three years in research on genetic engineering initiated by University of Edinburgh biologists seems to bring the more optimistic speculations on the potential benefits of these controversial techniques a step closer to reality. One of the most widely cited possibilities has been the production of insulin from a mammalian gene inserted into a bacterial phage or plasmid. Yet projects aimed at this kind of application, including the Edinburgh-ICI collaboration, are still very much at the research, and short of the development, stage.

The outstanding problem takes the form of a fundamental question in molecular biology: will the synthetic machinery of a bacterium lend itself to the production of a protein specified by a mammalian (or any other eukaryotic) gene? Morrow and his colleagues in the United States have succeeded in demonstrating that bacteria can transcribe eukaryotic DNA into RNA (*Proc. natn. Acad. Sci.*, **71**, 1743; 1974); but whether they can achieve the next step—the translation of the RNA into protein—is still not known. Furthermore, this is much the trickier step of the two, for while the genetic code is universal, the software for translating it into protein is not. Optimists believe that the first eukaryotic protein will be emerging from bacterial cells within the next year or two. Equally distinguished sceptics foresee that the highly specific molecular recognition and control systems governing protein synthesis will present serious problems.

Dr Kenneth Murray, whose work with his wife Noreen on phage lambda (*Nature*, **251**, 476; 1974) led to the ICI project, suspects that bacteria can be made to translate mRNA specified by eukaryotic genes, though perhaps rather inefficiently. One way in which he can conceive of overcoming specificity problems is by reducing the inserted DNA to the bare structural gene, as free as possible from regulatory sequences that would be unrecognisable to a bacterial synthetic system.

That raises the further technical problem of accurate excision of single genes from the donor DNA. The current burst of genetic tailoring took off from the isolation in 1972 of the bacterial restriction enzyme *Eco*RI, whose peculiar properties make it particularly suitable for the cleavage and reconstitution of heterologous DNA. But the very property of specific cleavage that makes the enzyme so useful also makes it inflexible: it will only cut a molecule where it wants to, and not necessarily where you want it to. Consequently one of the first requirements will be a range of enzymes with different specific cleavage sites. Researchers at Edinburgh and elsewhere are already at work on the accumulation of such a versatile set of molecular scalpels, but no-one is yet ready even to think in concrete terms about operating on such exotica as insulin genes.

With the recent emphasis on the possible hazards of genetic engineering research, safety has become a prominent issue. In fact, the experiments on phage lambda fall outside the forbidden categories I and II of the US National Academy of Sciences' cautionary statement, into the more amorphous category of experiments which "should not be undertaken lightly". One of the principal points which Murray has stressed in connection with the phage lambda system is its relative intrinsic safety. It does not carry drug resistance factors and is therefore susceptible to control, and has only been used so far with strains of *E. coli* to be found in the human gut. If the system comes into use on a commercial scale, Dr Murray is willing to envisage the development of bacterial protein factories custom-built to be incapable of infecting a human host.

In the meantime, precautions at Edinburgh include scrupulous sterilisation procedures. So far, the Murrays themselves have been the only people to have handled doctored phage particles. Not that they really believe that phage presents a serious threat to health; with the courage of their conviction, each Murray swallowed an experimental dose of 10^9 labelled phage —without either immediate ill effects, or traces of surviving organisms in the gut flora thereafter.

The injection of ICI funds will now, of course, make it possible to adapt a laboratory designed to contain pathogens, while at the same time the expansion of the project will mean the involvement of more personnel. Proper training of laboratory personnel will be emphasised since structural safeguards such as air-locks and protective hoods —both on the agenda—offer no protection from human carelessness.

The obvious advantage of an academic-industrial collaboration such as that between Edinburgh University and ICI is the pooling of intellectual resources not normally available to industry and practical resources not normally available to universities. There is one university department, however, which is uniquely placed from both points of view. The Department of Biochemistry at Imperial College London possesses a pilot plant for growing up to 60 kg of bacteria, as well as facilities for purifying large quantities of enzymes, and is now under the chairmanship of Professor Brian Hartley. Hartley and his collaborators have been working for the past few years on enzyme adaptations in bacteria (see for example *Nature*, **251**, 200; 1974). The most recent development in this work has been the use of genetic engineering techniques to transfer the genes specifying the enzymes between bacterial species. Hartley's plans for the biochemistry department include the recruitment of young molecular geneticists with a view to developing and exploiting these sophisticated techniques in the production of commercial quantities of enzymes tailor-made to the purposes of medicine and industry.

His approach differs from that of the Edinburgh team in a number of ways. For one thing, their project is based principally on the use of phage lambda as the vehicle for the transplanted genes, whereas Hartley's will involve both phage (where that seems advantageous) and bacterial plasmids. (This will bring the Imperial College project into NAS category I, so that out of respect for the moratorium it will have to be deferred, though probably not for long.) But there are also

THE bacterial enginering argument so far has been given most prominence in the United States and Britain, and it is likely that these two countries will be the first to formulate a set of guidelines, either as legal constraints or as informal codes of practice.

But the problem is worldwide, and the International Association of Microbiological Societies has recently set up an *ad hoc* committee to advise it on the hazards inherent in recently developed techniques of genetic engineering.

This committee will not attempt to duplicate the activities of either Professor Paul Berg and his committee in the United States or Lord Ashby's Working Party in the United Kingdom, according to the chairman, Professor S. W. Glover of the University of Newcastle-upon-Tyne. Rather it hopes to evaluate any recommendations made by these committees or at the international meeting planned to take place in California next February, and if necessary, to arrive at a set of constraints or guidelines which it can recommend to the IAMS for worldwide communication through the network of national societies affiliated to the IAMS.

Apart from Professor Glover, the committee consists of Dr E. Wollman (France), Dr A. Demain (US) and Professor G. Terui (Japan).

Business report

by Roger Woodham

FACED with two proposals for dealing with their company's liquidity crisis, employees of George Kent Ltd have voted in favour of an arrangement with the Swiss company Brown Boveri which would give Kent more cash to the tune of £6 million but leave effective control of the company in the United Kingdom. The name Kent on scientific instruments is well known in its own right in research laboratories, as is that of a company which it owns—Cambridge Scientific Instruments.

The result of this exercise in industrial democracy flew in the face of Mr Anthony Benn, Secretary of State for Industry, who preferred an arrangement whereby George Kent would be taken over by GEC, Sir Arnold Weinstock's company. But Mr Benn was also keen that Kent's employees should have a chance to make their views known and he now has to decide, in the light of the ballot, how to use the 24% of the Kent shares which the government owns when it comes to a vote among shareholders. If he decides to go along with the Brown Boveri plan it seems almost certain to be the one that will win the day.

Brown Boveri originally proposed that it should buy 53% of the Kent shares but it has now decided to be content with less than 50%, thus assuaging fears that control of George Kent would be lost to a foreign company. It also envisages Kent's scientific and medical instruments business being set up as a separate company owned by the mooted Brown Boveri Kent Ltd, and this is where some Kent employees part company with the Brown Boveri plans. The 600 employees of Cambridge Scientific Instruments voted in favour of the GEC plan to a man because they regard an independent instrument company as a doubtful starter because of its small size. Over George Kent as a whole, however, 75% of the 7,300 employees said yes to Brown Boveri.

This desire to run into the arms of of a Swiss-based multinational is almost certainly based on fears that 'rationalisation' after a takeover by GEC would threaten job security. By contrast there is little overlap between the activities of Kent and Brown Boveri, and the latter sees Kent as a useful marketing base in the United Kingdom. Indeed the prospect of access to France, Germany and Switzerland, where Brown Boveri sells some £780 million worth of equipment and where neither Kent nor GEC is particularly strong, is an added attraction from Kent's point of view.

major differences in both methods and aims. With his background in enzyme evolution and bacterial adaptation, Hartley expects to rely very much more heavily than the Murrays on the adaptive potential of the bacteria themselves. This might be used, for example, to produce enzymes stable enough to survive long periods of storage at room temperature. The first step would be to introduce the gene into *Bacillus stearothermophilus*, which thrives at high temperatures. If an environment could then be devised in which the bacterium's survival would be greatly enhanced by the production of large quantities of the new enzyme, the bacterium would be under considerable selective pressure not only to make the enzyme, but to evolve a structure for it that would be stable at high temperatures.

In a collaborative effort with the Microbial Research Establishment at Porton, Hartley has already achieved the purification to homogeneity of 20 enzymes from thermophilic bacteria, in quantities of 50 kg. The facilities are to be moved to Imperial College in January 1975, and the intention is eventually to produce enzymes for sale to industry. There is also the possibility of selling enzymes for research to other laboratories. Imperial College has at its disposal a potential wealth of restriction enzymes in the vast range of organisms grown there for antibiotic research. Isolation and purification of these enzymes will be an important part of the research programme.

The problem of maximising bacterial production of a particular enzyme for either commercial or research purposes may be another case in which the answer lies in the versatility of the bacteria themselves. In the case of phage lambda, there is a well known promotor system which it may be possible to commandeer. This enzyme system, together with the product of another phage gene, can override the controls which normally serve to prevent or limit the transcription of a given gene. Up to half the cell's synthetic capacity may in consequence be recruited to the production of a single type of protein molecule. In bacteria, however, it may actually be possible to increase the number of genes from which transcription of a given mRNA taken place. Hartley and his colleagues have recently demonstrated (*Nature*, **251**, 200; 1974) that bacteria often respond to metabolic demands for large quantities of a particular enzyme by duplication of the genes which code for it. Again, of course, this technique would involve manipulation of the environment and not the bacterium.

Hartley draws a categorical distinction between the prospect of getting bacteria to mass-produce enzymes to human specifications (science), and that of inducing them to make mammalian hormones such as insulin (science fiction). This is partly because of his own emphasis on bacterial evolutionary potential in the implementation of human designs. While it may not be too difficult to devise environmental exigencies that would force a bacterium into an unnatural need for an *outré* enzyme, it is extremely difficult to imagine how one might provide it with an incentive to produce insulin—although with elaborate genetic manipulation it may be possible. Hartley is not, however, among those who think translation will be a major problem, though he concedes it is the rate-limiting step. But in view of the extensive use already made in industry of microbial enzymes, not to mention long-term plans for solving the global food problem with microbial proteins, bacterial enzyme production may prove quite enough to be getting on with. □

Little Red greenery book

from our Soviet Correspondent

THE all-Union Botanical Society of the USSR has completed compilation of its "Red Book" of flora, listing some 20,000 forms in need of governmental protection, some 600 of which are in immediate danger of extinction.

In commenting on the report, *Pravda* predictably pays prime attention to those species which provide "irreplaceable" raw material, such as the Siberian and Korean cedars, and the Caucasian ironwood, and notes that such valuable species as the waterfall poplar, Vavilov's almond, and the Kolyma currant are apparently, already, irretrievably lost.

Nevertheless, the red book also includes plants which have so far found no economic use, including certain weeds "against which mankind has waged an agelong struggle". This would appear, at first glance, contrary to Soviet utilitarian theories, but, the commentator explains, the "dialectics of 'harm' and 'use' is in this case complicated and contradictory", and quotes the classic case of penicillin in order to justify the presence of the weeds. □

Musical chairs on Capitol Hill

by Colin Norman, Washington

FOR six days early in October the US House of Representatives put on a display of squabbling and infighting which would almost have done justice to Gladstone's Parliament during the stormy days of Irish 'reform'. One venerable Representative announced at one point that she would "fight to the death" to get her way, while another remarked that the House had lost its collective sanity. In the end, however, there were no open fisticuffs on the floor, as there were in Gladstone's day, and the whole affair got scant attention from the general press. But for science and technology, the titanic struggle on Capitol Hill holds some important implications.

The basis of the squabbling was a bold attempt to reform the House's antiquated committee structure—not exactly a heart-stopping issue, which is probably why it did not grab too many headlines—and the outcome can best be described as a standoff between the reformers and those who were hoping to preserve the *status quo*. But it will, at least, change the way in which the House handles a good deal of legislation involving research and development.

In short, the committee on Science and Astronautics, which has never exactly been a giant on the Congressional landscape and whose influence has been shrinking recently along with the decline in the space programme, is set to pick up some important new responsibility.

In addition to its present authority, which is essentially limited to the programmes of NASA, the National Science Foundation, the National Bureau of Standards and general science policy deliberations, the committee will pick up jurisdiction over all non-nuclear energy research and development, environmental research and development, civil aviation research and development and the Weather Service. In line with its new roles, the committee also gets a new title—the Committee on Science and Technology.

Certainly, it can be argued that the committee still cannot be called the focal point for scientific matters in the House since the really big spenders remain outside its jurisdictional patch. Defence research and development stays under the jurisdiction of the Armed Services Committee, for example, the Commerce Committee retains control over biomedical research, and the Joint Committee on Atomic Energy has lost none of its authority over nuclear matters. But the changes could turn out to be significant, particularly in regard to energy research and development.

First, by way of background, some remarks are in order on the functions of Congressional committees and the need for reform.

Committees in the House resemble a collection of mediaeval fiefdoms presided over by extremely powerful—and usually elderly—chairmen who have risen slowly through the ranks during their years of residence on Capitol Hill. The so-called seniority system requires that committee chairmanships (and the chairmanships of subcommittees) go to the longest-serving—and not necessarily the most able—committee members who belong to the political party which holds the majority of House seats.

Each committee has its own area of responsibility—thus, for example, the Armed Services Committee deals with legislation concerning programmes of the Department of Defense and the Agriculture Committee deals with agricultural legislation. When a bill is introduced into the House, it is referred to the appropriate committee, which usually assigns it to one of its subcommittees. Hearings are held on the bill, it usually gets amended or completely rewritten by the subcommittee before being passed on to the main committee, which can further amend it before it moves on to the full House for further amendment and final passage.

The committee therefore represent the centres of power since they shape the legislation which reaches the floor. Moreover, committee chairmen (and to some extent subcommittee chairmen) wield a tremendous amount of power over the system since they control the appointment of committee staff, who deal with the nuts and bolts of the committee work and frequently write the legislation themselves. And, equally important, the chairmen can kill off legislation with which they disagree simply by keeping it bottled up in their committees, never allowing it to be brought to a vote on the floor of the House.

One problem with the system is that it has been more than a quarter of a century since any serious attempt was made to alter committee jurisdictions, and areas of authority have consequently become considerably blurred. Take energy research and development, for example. The Committee on Science and Astronautics is deep into that subject since it has jurisdiction over the programmes of the National Science Foundation, but so is the Interior Committee, the Public Works Committee, the Commerce Committee and the Joint Committee on Atomic Energy. The net result is that there has been considerable jurisdictional turf fighting between committee members for control of energy research legislation.

Another effect of confused jurisdictions is that when a member of the Committee on Science and Astronautics, for example, writes a bill aimed at setting up a programme of, say, solar energy research, he shapes the legislation to give the programme to the National Science Foundation or NASA so that his committee gets jurisdiction over it. The result is that programmes don't always get assigned to the most suitable agencies.

Then there is the problem that Members of Congress often serve on several committees, which considerably dilutes their effectiveness.

So, all-in-all, the House of Representatives is not a terribly efficient place, and that's why two years ago a special bipartisan committee, headed by Richard Bolling, a skilled legislator from Missouri, was appointed to recommend some reforms.

Almost a year ago, the Bolling Committee came up with a batch of recommendations which immediately threw most of the committee chairmen into fits of apoplexy. It suggested a fundamental realignment of committee jurisdictions, that members could only serve on one major committee, and that the powers of the Speaker should be elevated.

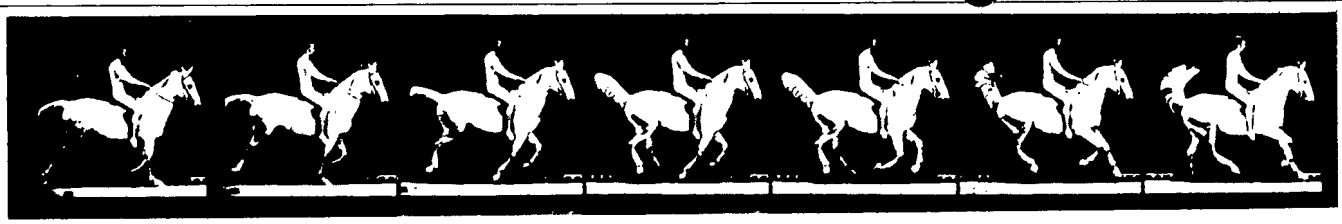
But by far the most controversial provisions it contained were the committee realignments.

For a start, the Bolling proposals would have stripped one of the most powerful committees—the Ways and Means Committee—of many of its functions, it would have split the Education and Labour Committee in two, and it would have abolished the Post Office Committee, the Internal Security Committee, and—to all intents and purposes — the Merchant Marine Committee.

Not surprisingly, a good number of committee chairmen who had reached the pinnacle of their political careers were not altogether happy about having their power suddenly stripped from them, and they persuaded the Democratic Caucus to appoint another committee to come up with some counter proposals.

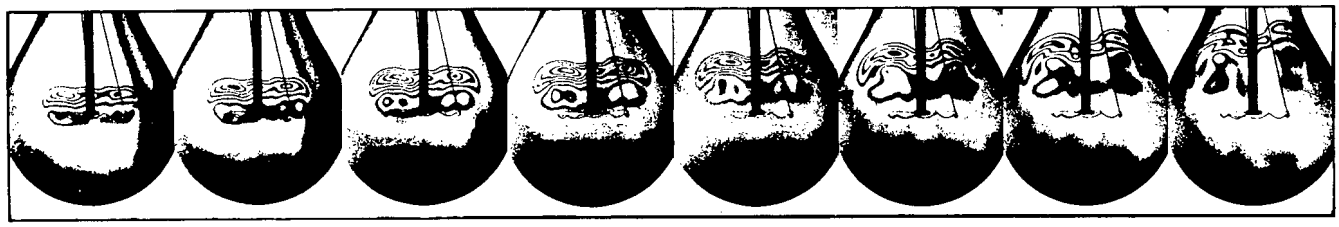
It was those recommendations which were adopted last week, with a few minor modifications, after six days of bitter debate which at times left the House leadership entirely confused.

As far as science and technology are concerned, the Bolling Committee had recommended that the Committee on Science and Astronautics should be given authority over all energy research and development, including



Fast workers

EADWEARD MUYBRIDGE (he thought the Anglo Saxon version more striking than plain Edward Muggeridge) was a Victorian photographer who left Kingston-on-Thames in 1852 to sail for America. There he was run over by a stage coach and tried for the murder of his wife's lover. He was declared insane and acquitted after a former employee had testified: "He was most eccentric in his work; he would not take a picture unless the view suited him." On the credit side, he made a stereoscopic record of the Modoc Indian war and toured Central America (as Edwardo Santiago Muybridge), later selling collections of 120 prints at 100 dollars the set, which was a close call to skinning the market at 1876 exchange rates. More importantly, Muybridge made photographic studies of movement which laid the foundations of a completely new high speed photographic industry (not to mention a completely new movement in European art). In 1880 he became the first person to photograph movement (using "the famous zoopraxiscope camera") and resynthesise the movement on a screen. A strip from this original sequence (above) was featured in an exhibition accompanying the eleventh international congress on high speed photography at Imperial College, London. The same congress was marked by an exhibition of applied photography at the Royal Photographic Society, where the very latest achievements of the high speed photographic industry included the series reproduced below. It shows the development of a convective flow in the gas filling of an electric bulb as the current through the filament is switched on. The photographs are from eight three-dimensional holographic images reconstructed from a sequence of double exposure holograms recorded on a single plate with a pulsed ruby laser. The pulse length was about 1 ms and the interval between pulses was 20 ms. The pictures were produced by the National Physical Laboratory, with whose permission they are reproduced. The Muybridge series appears by courtesy of the John Judkyn Memorial.



nuclear energy. But the powerful Joint Committee on Atomic Energy objected at such an intrusion on its patch, and the Science and Astronautics Committee eventually wound up with only non-nuclear energy research and development. But that is a distinct improvement over the present arrangement in which authority is split over a number of committees, and it also means that the new Science and Technology Committee will get jurisdiction over the non-nuclear programmes of the umbrella Energy Research and Development Administration.

The Bolling Committee had also recommended that the science committee be given "special oversight" over military R & D, which would have given it authority to make studies and recommendations on the Pentagon's programmes, but it wouldn't have had the power to write laws in that area. But Edward Hébert, the 74-year-old chairman of the Armed Services Committee would have none of that, and his monopoly over the Defense Department programmes remains unbroken.

Finally, the Bolling proposals would have given the Science and Astronautics Committee jurisdiction over virtually all the programmes of the National Oceanic and Atmospheric Administration.

But Mrs Lenore Sullivan, Chairman of the Committee on Merchant Marine and Fisheries didn't relish the idea of her committee disappearing from under her, and the upshot is a grotesque split with some atmospheric programmes going to the new Science and Technology Committee, while oceanography stays with the Committee on Merchant Marine. But at least it was agreed during the floor debate last week that the two committees will work together on oceanic and atmospheric matters.

As for environmental research and development, both the Bolling Committee and the Democratic Caucus committee recommended that the new Science and Technology Committee would gain responsibility for that entire area, and so it will.

The upshot, then, is that many science and technology matters in the House will now be consolidated into one committee, which should make for smoother operations in some areas. But it should be pointed out that the seniority system has survived completely untouched, and nobody had the temerity to alter the workings of perhaps the most powerful committee of all—the House Appropriations Committee, which deliberates on the budgets of the executive departments and agencies.

Bolling, at least, remains philosophical about the outcome of his attempts at reform. "It is a good start", he said last week, and indicated that he will try again next session.

Meanwhile, the Senate has for some time been making noises about taking a look at its own hopelessly confused committee structure, but so far has shown no signs of doing anything about it. It is far from clear at this stage, for example, which committees will get jurisdiction over the Energy Research and Development Administration, and science affairs are strung out over a whole range of Senate committees.

Since all bills must be approved by both the House and the Senate before they become law, the House reforms are clearly only half-way measures, no matter how good they are. □

news and views

Fluctuations in climate

THROUGH most of the first part of this century it was widely taken for granted that climate is essentially constant, apart from short-term fluctuations, some of which might involve shadowy cyclic changes. In fact, the global climate was at that time changing—a rather general warming and increasing moisture in continental interiors (apart from the Americas)—in ways that made life easier for most people in most places. Hence, there was little investigation of the phenomenon. Now, however, the decades of neglect have given place to widespread concern over climatic change. This is partly because there is some evidence of a global cooling, and a change in the rainfall trends also, setting in from the 1950s onwards. There is also a more pressing alarm over the many signs in recent years of an increased range of variability of climate from one year (or short group of years) to another. The world population is already so large that there is no margin for even occasional bad years —bad, that is, in the sense of lowered harvest yields in several of the world's principal grain-producing areas. Indeed, the world's grain reserves have been reduced each year since 1970: both 1972 and 1974 will rank as bad years in the sense just mentioned, but even in a good year, such as 1971, the reserves fell, and are now under a quarter of what they were before this decade.

It was with facts such as these in mind that US Secretary of State Henry Kissinger told the United Nations General Assembly in April 1974:

> "The poorest nations, already beset by man-made disasters, have been threatened by a natural one: the possibility of climatic changes in the monsoon belt and perhaps throughout the world. The implications for global food and population policies are ominous. The United States proposes that the International Council of Scientific Unions and the World Meteorological Organisation urgently investigate this problem . . ."

And, in similar vein, Lord Rothschild wrote recently:

> ". . . there are several subjects in which I regret that the Think Tank has not so far taken an interest: one of these is the effect of the possible changes in our climate on the life of the inhabitants of this island. It would, I believe, repay study."

Also, the Climatic Research Unit at the University of East Anglia in Norwich, of which I am director, whose financial plight over the first 2½ years of its existence has several times been mentioned in *Nature*, now has the funds needed to start its work—thanks to the generosity of the Wolfson and Nuffield Foundations in this country and the Rockefeller Foundation in the United States.

This issue of *Nature* carries three more in the series of contributions to knowledge on the variations and variability of climate which it has been publishing in recent years. Drs Wood and Lovett report on page 594 on the rainfall variations measured in Ethiopia over the last 70 years, and the records of major drought years since AD 1540, analysed in relation to the 11-year sunspot cycles. Their result highlights the great range of variation of the annual rainfall in that country within each 10 or 11-year period as likely to have more impact than the longer-term trends, though possibly made more serious by these. On page 592 Brown reports a new link between variations in the Earth's magnetic field at the time of sunspot minimum and the strength of the subsequent sunspot maximum; together with the climatic evidence such as that presented by Wood and Lovett this raises the possibility of the use of the Earth's magnetic field to forecast changing weather patterns five or six years ahead.

The third contribution, on page 582, is concerned with what can be learnt of the longer record of climatic behaviour from tree rings, in this case the year-rings in spruce (*Picea*) growing in southern Germany. Dr Schiegl uses deuterium measurements on the tree rings to show how an indication of a long history of annual mean temperature might be derived. This technique is one example of a rapidly increasing number of types and uses of 'proxy' data to extend and corroborate the climatic record for periods before the invention of most meteorological instruments.

H. H. LAMB

Mass extinctions in the fossil record

THE problem of what caused the extinction of particular groups of fossils continues to intrigue both palaeontologist and layman. Every schoolboy learns about the dramatic and relatively sudden extinction of the dinosaurs at the end of the Mesozoic Era, and many are the more or less ingenious hypotheses put forward to account for it. My own favourite relates the extinction to the relative decline of the gymnosperms or naked seed plants in favour of the flowering plants during the Cretaceous period. The surviving conifer and cycad representatives include many producing oils with renowned purgative properties, from which one is drawn ineluctably to the conclusion that the poor dinosaurs died of constipation! The trouble with all such hypotheses is their *ad hoc* character, devoted specifically to the dinosaurs. Viewed in a broader context, the dinosaurs are seen as but one of a whole series of animal groups, both terrestrial and marine, which died out at the end of the Cretaceous about 65 million years ago. An even more spectacular phase of mass extinction, affecting a majority of invertebrate and vertebrate classes both on land and in the sea, took place towards or at the end of the Permian some 160 million years earlier. It is no coincidence that the three faunally defined eras of Phanerozoic time, the Palaeozoic, Mesozoic and Cainozoic, are divided by these two so-called crises in the history of life.

Attempts to account for these, and lesser, phases of mass extinction can be grouped into two broad categories, involving phenomena either extrinsic or intrinsic to our planet. The extraterrestrial explanations have usually centred around the deleterious effect of high levels of cosmic radiation, leading to widespread destruction of organisms, either directly or by damaging genes and thereby preventing successful reproduction. Some have argued for episodic pulses of increased radiation compared with that operating

at present, for which there is no independent evidence; others have postulated that at times when the geomagnetic field was reversing the 'magnetic protection' of the Earth diminished for period of perhaps as much as a few thousand years, allowing more radiation to penetrate to the surface of the continents and oceans. Explanations such as these have fallen out of favour in the last few years for a variety of reasons. For example, they have not been able to explain why marine organisms have been affected at the era boundaries more than terrestrial ones, especially plants. Furthermore, in the ocean the intensity of radiation diminishes to a small fraction of its atmospheric value only a few metres below the surface.

Attention has now turned to intrinsic factors. Following the pioneer work of Valentine and Moores (*J. Geol.*, **80**, 167; 1972) who were the first to seek an explanation for the Permian extinctions by bringing together concepts derived from plate tectonics and modern ecological theory, attempts adopting a similar approach have been made recently to account for the 'crises' at the close of the Mesozoic and Palaeozoic.

Hays and Pitman (*Nature*, **246**, 18; 1973) have speculated along the following lines. In the late Cretaceous there was a huge marine transgression over as much as a third of the continents, when sea level was raised hundreds of metres as a result of uplift of the mid-oceanic ridges. This was a consequence of an acceleration in the rate of seafloor spreading for which there is apparently good independent evidence. The transgression was instrumental in inducing a more stable, equable world climate, which led to significant diversification of many animal groups including the dinosaurs and, in the marine realm, reef corals, rudistid bivalves and planktonic foraminifera. Highly diverse, stenotopic organisms in stable environments are vulnerable, however, to even slight environmental change. The pronounced marine regression at the end of the Cretaceous, related by Hays and Pitman to deceleration of seafloor spreading rates, induced an episode of increased thermal gradients, seasonal temperature contrasts and storminess, together with a significant change in oceanic circulation patterns. This sudden decrease in environmental stability was sufficient to cause widespread extinction, affecting the groups cited above and many others. The dinosaurs' unsatisfied need was not so much for laxatives as for winter woollies!

A significant relationship between the late Permian extinctions and fall of sea level is also inferred by Schopf (*J. Geol.*, **82**, 129; 1974). His survey disclosed that the number or marine invertebrate families halved from the beginning to the end of the period while at the same time the area of shallow epicontinental seas diminished by rather more than half. The lowering of sea level was probably a consequence of the creation of the supercontinent Pangaea as separate continents collided. When this happened the seafloor spreading rate diminished as a 'brake' was inevitably applied to plate movement. There is no satisfactory explanation of why sea level began to rise again in the early Triassic, although Pangaea did not begin to disintegrate, with the creation of a new spreading axis between Africa and North America, until the early Jurassic. In the earlier hypothesis of Valentine and Moores, the extensive late Permian extinctions were attributed partly to increased interfaunal competition as formerly isolated continental shelves were joined together, and partly to a reduction in environmental stability consequent upon the creation of one large continent from several smaller ones (thus, seasonal climatic contrasts would have increased). Schopf draws attention to another factor.

Ecologists recognise an exponential relationship between species number and area of the habitat occupied. Modern theory has it that animal populations in a given region are in a condition of dynamic equilibrium, with rate of immigration being balanced by rate of extinction. The theory has been applied with great success to oceanic islands, which form an excellent laboratory for ecologists. Small islands cannot be held to possess 'impoverished' faunas, as formerly thought; they can only support small populations and consequently extinction rates are higher. It is a big jump from species abundance in tiny islands to familial abundance in seas bordering whole continents, with rate of immigration being replaced by rate of origination through evolution, but Schopf is prepared to take this bold step. He can at least claim the support of a leading researcher in the new ecology (D. Simberloff, *J. Geol.*, **82**, 267; 1974), who has erected a model for the Permian diversity reductions and extinctions based on reasonable biological assumptions, which shows a good accord with Schopf's data. Thus merely reducing the area of the Permian shelf seas might be sufficient in itself to explain the increased rate of extinction, reaching a maximum at the end of the period.

An attractive feature of the two hypotheses outlined above is that they account for changes in organisms inhabiting a wide variety of habitats in a way which is consistent with modern ecological theory, and provide a satisfying correlation with independently deduced geological events of great significance. In both cases the fundamental control on the extinctions is likely to have been variation in heat flow from the mantle, affecting the density, volume and spreading rates of oceanic ridge systems. Though many important questions remain unanswered, an explanation along these lines is much less of a *Deus ex machina* than that involving variations in cosmic radiation.

A. HALLAM

Neural hypothesis of muscular dystrophy is flourishing

MANY workers in the field of neuromuscular diseases will feel that there is more evidence in favour of the neural hypothesis of muscular dystrophy than Professor Bradley's article[1] would imply. Although it is true that Harris and Marshall[2] did not find evidence of functional denervation in dystrophic mouse muscle, the earlier and contrary observations of McComas and Mrozek[3] have now been confirmed by Law and Atwood[4]. Fibrillation potentials, usually regarded as a sign of denervation, are also a feature of murine dystrophy[5]. Possible ultrastructural correlates of synaptic dysfunction have been found in the axon terminals of dystrophic mice in studies from two laboratories[6,7]. Even if Harris and Marshall were correct, these authors demonstrated nonetheless that the neuromuscular junctions of dystrophic mice were unusually susceptible to adverse conditions within the experimental milieu.

Dr Salafsky[8] was one of the first to transplant minced muscle between normal and dystrophic mice and, by controlled experiments encouraging reinnervation of the transplants, was able to study their contractile properties. The explanation offered by Dr Bradley is insufficient to account for all his findings. Of those muscle transplantation studies in which care was taken to permit reinnervation, two[9,10] have largely confirmed Salafsky's work and one[11] has not.

In the cases of human Duchenne dystrophy the evidence is mixed. Fibrillation potentials certainly occur[12] and the work of Desmedt and Borenstein[13] is cited as indicative that denervated muscle fibres become reinnervated. In contrast, a careful electron-microscopical study by Dr Andrew Engel and his associates[14] has not yielded any evidence of structural abnormality in the motor axon terminals. This result, and the finding of normal numbers of motoneurones at

autopsy[15], does not contradict the neural hypothesis as originally proposed[16]. Indeed, recent studies with A. Upton and P. Jorgensen, as yet unpublished, strongly suggest that it is possible for motoneurones to have 'silent' synapses. In patients with unequivocally neuropathic disorders it now seems that neuromuscular junctions may become inexcitable but still capable of transmitting a neurotrophic influence to the muscle fibres. If this interpretation is correct, it is not difficult to conceive of more severe dysfunction in which the synapses, although present, can no longer subserve a trophic action.

So far as the motor unit counting results are concerned, the necessary electrophysiological technique was first applied by McComas, Sica and Currie[17] to the extensor digitorum brevis (EDB) muscle. The choice of muscle was subsequently criticised on the grounds that the motor nerve was vulnerable to trauma, particularly in disabled patients such as those with dystrophy[18,19]. This criticism would seem unfounded if the recent reports[20,21] of normal numbers of motor units in Duchenne dystrophy are correct. Our own solution to the problem of trauma was to study patients with very early stages of the disease and to extend the technique to muscles other than EDB. These last results[22] are in keeping with the earlier ones. Since the full papers by Panayiotopoulos *et al.*[23] and by Ballantyne and Hansen[24] have yet to appear, it is difficult to be certain that these workers have achieved the refinements of the counting technique reported by Bradley. Scarpalezos and Panayiotopoulos[20] in their brief description claim to be able to detect very small muscle responses "overlapping at noise level" on single, rather than superimposed, oscilloscope sweeps. Not only is this feat a logical impossibility, but the value of the superimposition technique (employed by ourselves) in detecting very small evoked biological signals has been accepted ever since the pioneering experiments of Dawson[25]. As practised by our own group, the motor unit counting technique has proved itself as a sensitive and reliable means of detecting neuropathic disorders and it is gratifying that Ballantyne and Hansen[24] have obtained almost identical values for healthy subjects. It is important to add that the technique has recently been employed in a 'blind' study of 17 possible cases of malignant hyperthermia (unpublished work with B. A. Britt and W. Kalow) and has so far been completely accurate in predicting the status of the subjects.

I feel that the most direct evidence against a neural aetiology of dystophy is the parabiotic cross-innervation experiment of Douglas and Cosmos[26] in mice. The strongest evidence against a myopathic aetiology (though not necessarily for a neural one) is the exceedingly elegant chimaera study of Peterson[27]. At first sight contradictory, these obseravtions may be reconciled by taking into account the stage of development of the animal at which the foreign nerve was introduced to the muscle. Thus, it is possible that the very first contact between a genotypically dystrophic motoneurone and a genotypically normal muscle fibre in the embryo will commit the latter to a dystrophic growth thereafter; if so, any later experimental manipulations with innervation would be ineffective.

Regardless of the true state of play, the neural hypothesis has successfully drawn attention to the possibility of deranged trophic mechanisms being present in neuromuscular disease. Whether or not the hypothesis is eventually shown to be correct, it will have served a useful function if it advances our understanding of dystrophy or brings a cure one step closer.

A. J. McCOMAS

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Drilling in the Antarctic

from Peter J. Smith

So much attention is given to the large-scale geological programmes, such as the Deep Sea Drilling Project, that there is a tendency to overlook the more modest investigations which face technical and logistic problems scarcely less formidable. A good case in point is the three-nation programme of drilling in the McMurdo Sound region of Antarctica. By the late 1960s it was clear that if significant progress were to be made in investigating the geology, geochemistry, glaciology, lake-bottom stratigraphy and thermal characteristics of the dry valleys west of McMurdo Sound, it would be necessary to supplement surface studies with borehole data. Accordingly, several drilling proposals were submitted to the National Science Foundation which coordinated all acceptable proposals into a single programme with participating scientists from the United States, Japan and New Zealand. Thus was born the Dry Valley Drilling Project (DVDP), since expanded to include Ross Island and McMurdo Sound itself.

The original aims of the DVDP as described by McGinnis *et al.* (*Antarctic J.*, **7**, 53; 1972) included studies of palaeontological and volcanic evolution during the poleward migration of Antarctica, palaeomagnetic reversals, the geochemistry of polar desert soils and permafrost, heat flow, the hydrogeology of ice cap margins and the global tectonic significance of the McMurdo volcanics, although it is already clear that the first of these objectives will not be met. An initial exploratory season was envisaged, followed by three drilling seasons involving a minimum of 10 holes. During the exploration in the 1971-1972 summer season electrical depth soundings were taken to determine permafrost thickness and the nature of the material beneath the

permafrost, seismic refraction profiles were run in lake basins to establish the relief and structure of bedrock and overburden thicknesses, and a regional aeromagnetic survey was made to provide structural control. In addition, environmental monitoring (physical and microbiological) was begun and will be continued throughout the project.

Drilling began during the 1972-1973 season with two holes through the volcanic rocks of Ross Island; and preliminary geological and geochemical results from these sites (boreholes 1 and 2) were described about a year ago (*Antarctic J.*, **8**, 157; 1973). During the 1973-1974 summer, seven more holes were drilled in the Ross Island volcanics (borehole 3 adjacent to hole 2) and in the dry valleys at Lake Vanda (boreholes 4 to 9); and a series of reports on this work has just been published (*Antarctic J.*, **9**, 125; 1974).

According to Kyle and Treves the cores from holes 1, 2 and 3 reveal that the geological history of Hut Point Peninsula (Ross Island) is much more complex than the surface geology suggests, which is itself sufficient vindication of drilling. The oldest unit penetrated by holes 2 and 3 is a 200+ m thick pile of hyaloclastite representing early eruptive events that took place below ice or water; in other words, an early stage of marine volcanism involved the construction of a hyaloclastite pedestal which may have impinged on a thick ice shelf covering the Ross Sea more than 1.2 million years ago. The higher lavas, on the other hand, are apparently subaerial flows and pyroclastic units which represent a single differentiation series starting with olivine–augite basalts, working through augite–kaersutite basalts and ending with kaersutite hawaiites, although the phonolites on an adjacent hill may well be more extreme differentiates of the same magma chamber.

The cores from the dry valleys not unexpectedly comprise glacial and marine sediments, although two of the holes also penetrated the crystalline basement. Both sedimentary and igneous cores are still under laboratory investigation so few geochemical and mineralogical results are available. But Tarii reports that stable isotope studies have already revealed the sources of core ice; in Lake Vanda, for example, most of the present water apparently originates as fresh water whereas deeper sedimentary layers are still under the influence of sea water. Also Gumbley *et al.* have begun to use the upper few metres of sediment from Lake Vanda to trace the lake's Late Quarternary history.

The DVDP can already be credited with the resolution of at least one long-standing disagreement. Over a decade ago, Armitage and House (*Limnol. Oceanog.*, **7**, 36; 1962) discovered that although Lake Vanda lies in a region where the mean air temperature is −18° C, it has a bottom water temperature of +25° C. This led Armitage and House, and later Angino *et al.* (*Sci. Bull.*, **45**, 1097; 1964), to suggest that below the lake there are either high geothermal gradients or hot springs.

Wilson and Wellman (*Nature*, **196**, 1171; 1962) ruled out hot springs on the grounds that the measured isotherms in the lake are nearly horizontal. Not only are hot springs unlikely in Antarctica because the great thickness of frozen ground precludes abundant groundwater; they argued that the entrance of springwater into the lake in conjunction with any possible hot spring would produce a much more complicated thermal pattern. Instead, they developed a theory of solar heating in which solar energy penetrates the lake's ice cover (found to be extremely transparent) and is absorbed in the water below. In support of this view, Wilson and Wellman pointed to the extreme clarity of the water and to the decrease in temperature gradient with depth (which implies a heat 'source' in the water itself). Heat flow measurements in the upper 30 cm of lake sediment also seemed to show that heat is flowing from the water to the sediment. But Ragotzkie and Likens (*Limnol. Oceanog.*, **9**, 412, 1964) produced precisely the opposite result from similar measurements and therefore attributed the high bottom temperature to a combination of solar heating and high geothermal gradient.

Wilson *et al.* have now resolved this question by making thermal measurements in DVDP hole 4 which penetrated the crystalline basement below Lake Vanda. The temperature in the basement 15.5 m below the lake bottom is consistently 0.48° C lower than that in the sediment 0.5 m below the lake bottom. The corresponding temperature gradient (average 0.032° C m^{-1}), combined with estimates of thermal conductivity, shows that Lake Vanda is losing heat downwards at a rate of 0.5–1.0 cal $cm^{-2}s^{-1}$, thus convincingly supporting the view that geothermal heat is not the reason for the lake's high temperature.

Corvine cannibalism

from our Animal Ecology Correspondent

ARGUMENTS have been raised for years about the functions and consequences of territories to animals. Since the ultimate restraint to population increase is availability of food, one might expect the relationship of territory size to food to be both positive and linear. For some species, mostly herbivores, this may be true or nearly so. But often for both carnivores and herbivores territory size is unrelated to food supply (Watson and Moss, in *Animal Populations in Relation to their Food Resources*, 167, Blackwell, Oxford, 1970). Hinde points out that territories have complex functions with consequences both harmful and advantageous to an individual's chance of breeding success (*Ibis*, **98**, 340; 1956). Simple answers cannot be expected to complex questions.

In a well designed series of field experiments on carrion crows in north-east Scotland, Yom-Tov added extra food to the environment to ascertain if there was a direct relationship between territory size, food supply and breeding success (*J. Anim.Ecol.*, **43**, 479; 1974). There was circumstantial evidence that there was no absolute food shortage during the breeding season. Food in the form of hens' eggs and dead hens' chicks was placed near to artificial trees both within and without established crow territories. This treatment failed to increase the breeding density of adults. But one egg and five chicks offered daily to a group of fourteen breeding pairs in the close proximity of their nests resulted in a significantly higher survival rate of nestlings although there was no difference in clutch size. At fledging an average of 2.3 young had survived in the experimental nests compared with 1.1 in the controls.

The other effect of food added daily from the start of the year was to significantly shift the date of the start of laying, bringing it forward by 5 days. Earlier layed clutches were almost twice the size of late clutches, but the

Eltanin bailed out

To oceanographers the name Eltanin probably stands second only to Glomar Challenger. From 1962 to 1972 this vessel carried out geological, geophysical, geochemical, biological and meteorological researches which covered some 80 per cent of the southern ocean between 35° S and Antarctica. Then a $1.5 million budget cut in the US Antarctic Research Program ended its active work.

But now the Eltanin is about to begin a new five-year programme on Antarctic research as a result of an agreement between the United States and Argentina. The ship, renamed Islas Orcadas and operated from Buenos Aires by the Argentine navy, will carry out joint scientific expeditions with support from the National Science Foundation and the Argentine National Antarctic Directorate.

number of fledglings produced per nest was about half. So increased winter food affected date of laying but not, *per se*, the number of fledglings produced per nest.

How does extra food increase the survival rates of nestling crows? Predation of the eggs and desertion by the adults were the main factors responsible. Krebs has pointed out that artifically increased winter food has no effect on the number of territorial breeding pairs of great tits during the following spring (*Ecology*, **52**, 2; 1971). Those nests that were closer together than 45 m suffered 12% more predation (mostly by weasels) than did those nests spaced over 45 m apart. Carrion crows are their own chief predators—they prey to a significant extent upon the eggs and nestlings of their own kind. With cannibalism as the regulatory mechanism, the ultimate limiting factor for the number of nestlings reared is the dispersion of food within the territory. An abundance of food close to the nest means that the clutch is left unattended for shorter intervals than if the food is widely scattered. To remain near the nest breeding crows limit their territory size. The lower limit to territory size depends upon the amount of food available and the upper limit depends upon its dispersion and the ability of the pair to defend its nest.

Cyclic AMP and pattern formation

from Paul Epstein

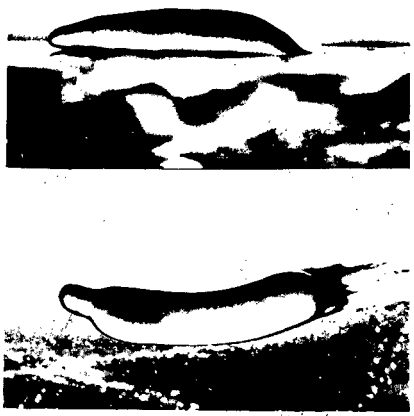
By staining with fluorescent antibody specific for cyclic AMP, Pan *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 1623; 1974) have been able to observe the distribution of cyclic AMP in both the unicellular amoebae and the multicellular pseudoplasmodium of *Dictyostelium discoideum* and several related species of cellular slime mould. Cyclic AMP was first shown to be important in the life cycle of *D. discoideum* when it was isolated as the naturally occurring acrasin, or chemotactic agent by which the vegetative amoebae signal each other to aggregate (Barkley, *Science*, **165**, 1133; 1969). Now the studies by Pan *et al.* suggest that cyclic AMP is important not only for aggregation, but for determination, or pattern formation, as well.

In the cellular slime moulds, once the aggregation process is completed, the amoebae form a pseudoplasmodium, or grex, which, depending on environmental conditions, may migrate for an indefinite period before beginning to differentiate into two morphologically and functionally distinct cell types—spore cells and stalk cells. By staining with vital dyes, Bonner (*The Cellular Slime Molds, second ed.*, Princeton University Press, Princeton, New Jersey, 1967) showed that the cells in the anterior third of the grex become stalk cells, whereas the cells in the remaining posterior portion become spore cells.

The cells in the grex are not predetermined, however, since a grex which is sliced transversely can form two normal fruiting bodies, containing the normal proportions of spore and stalk cells (Raper, *J. Elisha Mitchell scient. Soc.*, **56**, 241; 1940). This observation indicates that the cells can recognise their position within the grex, and somehow alter their developmental fate accordingly.

How do the cells know their position in a field? One theory is that they respond to gradient(s) of concentration of small molecule(s) (Wolpert, *J. theoret. Biol.*, **25**, 1; 1969). More recently, McMahon developed a theory of pattern formation using *D. discoideum* as a model, which holds that a sharp concentration boundary of a small molecule can develop through its regulation by contact-sensing molecules on the plasma membranes of cells in the field (McMahon, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2396; 1973). These regulatory molecules would be the macromolecules which synthesise and degrade the small molecule. In this case, the polarity of the morphogenetic field is determined by the boundary of the small molecule, which in turn is determined by the distribution of contact-sensing molecules.

Although one theory invokes a gradient and the other a boundary, both models require non-uniform distribution of a small molecule to determine the pattern of development. The results of Pan *et al.* suggest that cyclic AMP might be one such small molecule which determines pattern formation in *D. discoideum*. Pan *et al.* showed that as the pseudoplasmodium was allowed to migrate, a distribution of cyclic AMP arose such that the higher concentration was found in the anterior, pre-stalk region. In some cases, they observed an abrupt boundary of cyclic AMP concentration between the anterior and posterior portions of the grex, as theorised by McMahon.



Migrating grex of *Dictyostelium discoideum* seen from the side and from above. From *Developmental Biology* by Nelson Spratt.

It is interesting to consider two earlier observations which bear on pattern formation and differentiation in *D. discoideum*, and to see how they fit with the idea that pattern formation in the grex is determined by the distribution of cyclic AMP. Bonner (*Proc. natn. Acad. Sci. U.S.A.*, **65**, 110; 1970) subjected isolated amoebae to high concentrations of cyclic AMP and found that they developed into stalk cells. From this observation, he concluded that cyclic AMP functions as an inducer of stalk cell differentiation. The observation of Pan *et al.* is consistent with this conclusion.

The other observation was made by Raper (*J. Elisha Mitchell scient. Soc.*, **241**, 1940), who sliced a pseudoplasmodium transversely into four sections. Section one, the anterior tip, could be made to migrate for various times. If induced to begin fruiting body formation immediately, without any resumption of migration, this section developed into an extremely abnormal fruiting body, bearing very few or no spores, and an excessively heavy stalk. If 3–6 h of migration were allowed before fruiting formation began, the resulting structure was less abnormal but still bore fewer spores and more stalk. If 24 h of migration were allowed, the tip gave rise to a completely normal fruiting body, with the correct proportion of spore and stalk cells. Hence, Raper demonstrated that the cells at the anterior tip of a pseudoplasmodium are determined to become stalk cells, and that this determined state is altered progressively as the tip migrates.

In accord with this observation, Pan *et al.* found that the distribution of cyclic AMP in the grex becomes more apparent as it migrates. Likewise, McMahon, in his theoretical treatment, showed the boundary of cyclic AMP concentration arising near one end of the field, and moving along the field with time. McMahon calculated that by about 3–5 h the boundary becomes stabilised at a position one-third of the way down the field; that is, at the position which would give rise to the normal proportion of spore and stalk cells.

Although substantial proof is required before one can state that cyclic AMP is responsible for pattern formation in *D. discoideum*, the results of Pan *et al.* at least suggest such a function. If further studies provide proof, this would represent the first identification of a small molecule responsible for pattern formation.

Clustered genes and non-transcribed spacers

from Benjamin Lewin

MOST eukaryotic genes are probably present in only one copy for each haploid genome. Two notable exceptions to this rule are provided by the genes coding for histone proteins and those which specify ribosomal RNAs: here many genes, apparently identical for ribosomal RNA genes and with little variation in the histone genes, are organised in a cluster in which each transcribed sequence seems to be separated from the next by a non-transcribed spacer region. The structure and function of the spacer are presumably relevant to the control of gene activity, and perhaps to the problem of the suppression of variation in the multiple copies of each transcribed sequence.

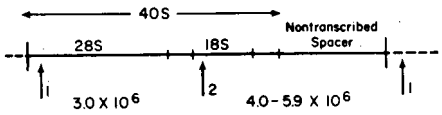
During oogenesis in *Xenopus laevis* the nucleoli which contain ribosomal DNA are amplified to become a major cell component. The ribosomal DNA can thus be isolated in bulk and has been the subject of much work on sequence organisation. This DNA was recently used as a substrate for the *Eco*RI endonuclease of *Escherichia coli* by Morrow *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **71**, 1743–1747; 1974) who found that each repeating unit (transcribed plus non-transcribed) suffers two cuts. When *X. laevis* rDNA molecules of about 50×10^6 daltons were treated with excess *Eco*RI enzyme the principal fragments had weights of 3.0×10^6 and 4.2×10^6 daltons; prominent among the minor fragments were two of 3.9×10^6 and 4.8×10^6 daltons. These products were annealed with DNA of the plasmid pSC101 (which is cleaved at a single site, generating a linear molecule from its circular genome) to give plasmid–rDNA recombinant molecules that were then replicated by growth in *E. coli*.

When the recombinant plasmid DNAs were isolated from several bacterial cell lines and analysed by cleavage with the *Eco*RI restriction enzyme, each recombinant plasmid gave fragments with sizes typical of the linear molecule of pSC101 and of the cleavage products of *Xenopus* rDNA.

By hybridisation with 18S and 28S *Xenopus* rRNA, Morrow *et al.* showed that the hybrid plasmid CD4, which contains both the 3.0×10^6 and 4.2×10^6 cleavage fragments, anneals equally well with both rRNAs; the CD18 plasmid containing only the 3.0×10^6 dalton fragment anneals principally with 28S rRNA; while the DNAs of plasmids CD30 and CD42, which contain the 3.9×10^6 and 4.2×10^6 dalton fragments respectively, react mostly with 18S rRNA. These results suggest that the tandem repeats in the rDNA are heterogeneous, each consisting of the 3.0×10^6 dalton sequence (which contains the 28S rRNA sequence) and the 3.9×10^6 or the 4.2×10^6 dalton sequence (containing the 18S rRNA sequence and presumably differing by the presence or absence of an extra sequence; other variations in this region are suggested by the existence of other size fragments).

Since the transcribed region of *Xenopus* rDNA takes only one form, heterogeneity in sequence must be ascribed to the non-transcribed spacer region, a conclusion supported by the report of Wellauer *et al.* in the *Proceedings of the US National Academy of Sciences* (**71**, 2823–2827; 1974). By cleaving *Xenopus* rDNA with the *Eco*RI enzyme, they were able to isolate two classes of fragment on agarose gel electrophoresis. Every preparation contains a prominent band of 3.0×10^6 daltons and several bands are present in lesser amounts which vary from 4.0 to 5.8×10^6 daltons. To decide whether the heterogeneity of the larger bands exists within single genomes or reflects differences between animals, they examined the rDNA from a single nucleolus organiser; this contained at least three of the larger size classes as well as the 3.0×10^6 dalton band. The repeat length of transcribed plus non-transcribed *Xenopus* rDNA is about 8×10^6 daltons, clearly implying that the repeats must be heterogenous in length (since otherwise the total length of fragments would equal rather than exceed the repeat length). When the duplex lengths of the fragments were measured by electron microscopy, the smallest proved to have a homogenous size of 3.0×10^6 daltons and the number of these small fragments equalled the sum of the number of the larger fragments. Each repeat therefore seems to comprise one 3×10^6 dalton region and one of the larger regions.

Secondary structure mapping has previously been used by Wellauer and Dawid (*Proc. natn. Acad. Sci. U.S.A.* **70**, 2827–2831; 1973) to order the sequences for ribosomal RNA in the precursor of the mature rRNA (see Lewin, *Nature*, **250**, 619–621; 1974). When RNA is spread for electron microscopy, hairpin loops may form between short complementary sequences and the position of each loop along the linear molecule provides a map of it. The 28S rRNA has a characteristic map present at the 5′ end of the precursor and the 18S rRNA is characterised by an absence of loops. Wellauer *et al.* now extend this technique to DNA which has been denatured to single strands. The small 3.0×10^6 dalton fragment cleaved from *Xenopus* rDNA by the *Eco*RI enzyme is homogeneous in structure; it seems to result from cuts made by the enzyme about 0.3×10^6 daltons into the 28S rRNA sequence and about 0.2×10^6 daltons into the 18S rRNA sequence; this fragment thus represents the starting region of the rRNA precursor, lacking the immediate 5′ end. The other fragment generated by these two cuts has one end devoid of structure, presumably corresponding to the 18S rRNA sequence, with the other end displaying the structure typical of the very beginning of the 28S rRNA structure.



Summary by Wellauer *et al.* of structure of an rDNA repeat unit.

The central regions of all larger fragments show a related structure, but vary in length. It is therefore the non-transcribed spacer region in which the heterogeneity resides and the formation of heteroduplexes suggests that the shorter large fragments represent deletions of sequences present in the longer large fragments. How this heterogeneity relates to the functions of the nucleolar rDNA is not yet known; one reservation about the interpretation of these results, however, is that the source of the rDNA is the amplified material of the oocyte and it remains to be proven whether the same situation is found in the somatic cell.

Analysis of histone genes is at a less advanced stage than that of ribosomal RNA genes. Each of the five histone proteins seems to constitute a unique amino acid sequence in any species; and the hybridisation analysis of Weinberg *et al.* (*Nature*, **240**, 225–228; 1972) suggested that their messenger RNAs are coded by genes repeated some 500–1,000 times in the genome of the sea urchin *Psammechinus milaris*. Because of the multiple codons representing each amino acid, identity of protein sequences need not imply identity of the repeated nucleic acid sequences coding for them, but the hybridisation analysis suggests only limited heterogeneity. The histone messengers of another sea urchin, *Lytenichus pictus*, have been separated by gel electrophoresis into a small number of groups by Grunstein *et al.* (*Cold Spring Harb. Symp. quant. Biol.*, **38**, 717–724; 1973) and a fingerprint analysis of one of these messengers is consistent with the idea that its sequences are fairly uniform.

The histone genes of *Psammechinus milaris* band on CsCl gradients at a density only slightly greater than that of bulk DNA, but Birnsteil *et al.* (*Proc.*

natn. Acad Sci. U.S.A., **71**, 2900–2904; 1974) now report that they can be isolated by their ability to bind preferentially to actinomycin C_1 which causes them to form a low density band. The band isolated in this way hybridises well with a preparation that contains the messengers for four histones (mRNA activity for histone F_1 has not yet been demonstrated); since each of the four histone messengers shows a virtually identical reaction with the fractions of DNA separated on the CsCl–actinomycin gradient, it is likely that the different histone genes are intermingled rather than clustered in four individual groups.

Comparison of G+C content of histone DNA and RNA, and the results of shearing and of melting histone DNA all suggest that the histone gene cluster also contains other sequences that are poorer in G+C content. The release of the histone-coding from the other sequences only by shearing suggests that both components are integral parts of the gene cluster. The melting curve of renatured DNA from the preparation implies the presence of some heterogeneity in sequence, probably in the sequences which do not code for histones. Whether the component poor in G+C represents a non-transcribed spacer region analogous to that of the rDNA gene cluster is not of course revealed by present data but this is one of the functions that can be imagined for it.

Two by two

from D. H. Jennings

IT is rare these days to attend a conference covering the whole spectrum of biology. But the Society for Experimental Biology mounted such a conference—on symbiosis—at Bristol on September 2–6. By retaining the original de Bary definition, namely that symbiosis is the association of two different organisms, it was possible to have all shades of interest from molecular to whole organism biology represented. Thus, ecologists, though not contributing directly, could find much to interest them. Indeed, two contributions were particularly seminal for ecological studies. Fricke (Max-Planck-Institut, Seewiesen) indicated how behavioural studies of organisms in the Red Sea can provide an understanding of animal interactions in nature thus adding flesh to the bare bones of numerical population studies. Cox (Kings College, London) discussing his work on intraerythrocytic parasites, pointed out that population biologists cannot continue to think about particular species without considering their parasites. He showed how susceptibility to various virus diseases can be dramatically modified by the presence of parasites.

There was much emphasis on how the invading symbiont avoids attack by its potential host. Terry (Brunel University) and Smithers (National Institute for Medical Research, Mill Hill) presented their elegant studies on the evasion of the immune response by *Schistosoma* which can survive by its ability to acquire host molecules. Muscatine (University of California, Los Angeles) provided data indicating the recognition by *Hydra* of the appropriate *Chlorella* cells takes place after engulfment at the stage when the algal cells move to the base of the digestive cells. Studies on symbiosis in *Paramecium* are now also beginning to yield relevant information with respect to bacteria (Preer, University of Indiana, Bloomington) and *Chlorella* (Karakashian, Max - Planck - Institut, Wilhelmshaven).

In spite of the decision to use the de Bary definition, there was much discussion of what is meant by the term 'symbiosis'. Mortimer Starr (University of California, Davis) produced a new classificatory scheme which has the virtue of both clarifying thinking about the phenomenon and indicating where further experimental work is required—to decide, for example, whether a symbiotic association is mutualistic or obligately parasitic. In this respect, Smith (University of Bristol) put those interested in lichens further in his debt by his careful analysis of the present data in the field which indicate that there is no detectable benefit to the algal partner. Also Coffey (Trinity College, Dublin) presented further information about the rust fungi, once thought to be the classic example of obligate pathogens in plants but which can now be grown in culture.

Many problems are emerging for the biochemists, particularly on how a symbiont affects the physiology of the host which it has invaded. Biochemists should be impressed by *Aplysia* which does a far better job than they of isolating chloroplasts. There is no doubt, as shown by Trench (Yale University), that these chloroplasts are fully functional, though the relationship between these organelles and the animal cytoplasm in which they reside is not the same as that which exists in the intact cell from which the chloroplasts are extracted.

But biochemists and molecular biologists have other reasons for being interested in symbiosis. Chloroplast symbiosis is, in a number of ways, the living expression of how eukaryotic organelles are believed to have arisen, namely by successful invasion of a primitive glycolytic prokaryote by oxygen utilising (to give mitochondria) and photosynthesising (to give chloroplasts) prokaryotes. Though the theory has a respectable age, Lynn Margulis (University of Boston) has done the most recently to bring it up to date. In a debate on the theory she propounded her views with infectious enthusiasm. But though most of the audience were believers, Raff (University of Indiana, Bloomington) in a careful comparison of bacteria and mitochondria showed that their faith may be blind.

Five hundred million years ago in Wales

from I. Strachan

ABOUT half of those attending the symposium organised by the Palaeontological Association of London on the Ordovician System (September 17-20) had spent the previous week touring Wales looking at the classic geological sites of Arenig, Llandeilo, Caradoc and Bala.

Much of the material presented at the meeting in Birmingham was factual and included new information as well as regional summaries, but there was also a considerable questioning of current dogmas. F. J. Fitch (Birkbeck College, London) and others speaking on Ordovician geochronology pointed out the possible sources of error inherent in radio-isotopic dating, particularly in Lower Palaeozoic rocks, and stressed the need for the use of standard reference scales (such as that produced by the Geological Society of London in 1964) so that data from different sources could be accurately compared. They concluded that a length of 65 Myr (between 510 and 445 Myr ago) was reasonable for the Ordovician, compared with 70 Myr for the preceding Cambrian and 40 Myr for the succeeding Silurian.

Given this broad time span, it was not surprising that the details of the regional pictures presented by later speakers should show some differences. The biostratigraphical problems were analysed in various groups of fossils. W. B. N. Berry (University of California) related his graptolite faunas to warm and cool water masses using a palaeogeographical reconstruction with the land all on one hemisphere. M. Lindström (University of Marburg) felt that the conodont faunas agreed in provincialism with the graptolites but C. R. C. Paul (University of Liverpool) found distinct North American, Baltic and Mediterranean cystid faunas only up to the lower part of the Caradocian, after which time provincial boundaries became fuzzy. This he related to closing up of ocean basins

during late Ordovician plate movements.

He also discussed the functional aspects of respiration in cystids to critically low oxygen levels in shallow tropical seas. This linked the palaeogeographic approach to the climatic one which was discussed by N. Spjeldnaes (University of Aarhus, Denmark). The now well known glacial deposits of the Moroccan Anti-Atlas were beautifully illustrated by J. Destombes (Service géologique, Rabat) and other speakers referred to climatic cycles in the Ordovician which influenced the occurrence of various groups of fossils. Changes in faunas could be caused as much by climatic changes as by migration between continental plate margins and the disentangling of plate movements through the whole length of the Ordovician needs much further work.

None of the formal papers included any discussion of the boundaries of the Ordovician, most speakers making it clear whether they regarded the Tremadocian as Cambrian (as in English usage) or Ordovician. But the large gathering of experts from so many countries, including the Soviet Union, provided the opportunity for meetings of the Commission on Stratigraphy of the International Union of Geological Sciences. Several recently formed subcommissions and working groups of the commission will have a great deal to do with correlation of strata between countries and continents. Differences of interpretation were much in evidence during the closing general discussion in Birmingham, particularly between those based on groups of fossils traditionally used, such as brachiopods and trilobites, and those which have come into prominence in the last few years, such as conodonts. Some of the ideas were certainly provocative and augur well for the future liveliness of the subject. As an eminent worker on Palaeozoic gastropods put it to close the meeting—a snail never gets anywhere unless he sticks his neck out.

Expanding wings in moths

from our Insect Physiology Correspondent

EVERY lepidopterist knows that emerging butterflies and moths go through a characteristic sequence of wing-folding movements, with wing expansion and general hardening of the cuticle, before settling down into their normal resting posture; and that the force for wing expansion is the hydrostatic pressure of the blood generated by strong contraction of the abdomen. Truman and Endo (*J. exp. Biol.*, **61**, 47; 1974) have now elucidated the complex of physiological factors engaged in this familiar phenomenon.

It was shown by Fraenkel many years ago that if the newly moulted blowfly is obliged to continue burrowing through the soil, it remains soft with the wings and body unexpanded for many hours. Only when it makes its escape is the cuticle expanded, hardened and darkened. It was generally assumed that this hardening process must be controlled by a hormone, but that was demonstrated by Cottrell only in quite recent years and confirmed by Fraenkel and Hsiao, who named the hormone in question 'bursicon'. It is liberated from the brain and other ganglia of the nervous system.

Truman and Endo found that the emerging adult of the tobacco hawkmoth *Manduca* behaves in the same way. It likewise pupates in the soil, and so long as the emergent moth is confined it shows intense digging behaviour, and the spreading of the wings is suppressed, sometimes for 24 hours. The stimulus which maintains the inhibition comes from the head: decapitated moths spread their wings immediately, regardless of whether they are confined. Moths decapitated at the very moment of eclosion, however, show neither wing inflation nor wing folding. But if decapitation is delayed until five seconds after emergence, the full wing-spreading behaviour occurs.

This effect of the brain is not dependent on the nerve supply; it is the result of the neurosecretory 'eclosion hormone': a brain removed from the pupa and implanted into the abdomen of a decapitated pupa is wholly effective in evoking complete wing spreading. The suboesophageal ganglion is also necessary for wing spreading; but this seems to be a neural effect on movement. The hormone bursicon, set free from the abdominal nerve cord, will cause neither wing inflation nor wing folding; it has two important functions: it serves to plasticise the cuticle (as was shown by Cottrell in the blowfly) and it sets in motion the hardening of the cuticle which, in turn, apparently dictates by feedback to the central nervous system the timing of the wing-folding movements.

In brief the eclosion hormone, discovered by Truman a few years ago, not only initiates emergence, but also wing spreading, which is brought about by a central motor programme involving neural and hormonal stimuli and some degree of sensory feedback.

Plant conifers and lose water

from Peter D. Moore

A KNOWLEDGE of the rate of water loss from a forest is essential both for forest water budget calculations and in the management of water catchments. Many comparative studies have been undertaken to provide such information, on forest stands and on other vegetation types. Differences in water loss from various types of vegetation are explicable in terms of transpiration rates, in the effect of vegetation upon evaporation direct from the soil by providing insulating layers of varying thickness and spatial structure, and in the interception of precipitation and its subsequent evaporation directly from leaf surfaces.

The quantification of these parameters, however, is extremely difficult. One of the most useful approaches has been the development of detailed models which attempt to account for all of the microclimatic and spatial variables, each of which can be determined individually (for example, Monteith, *Symp Soc. exp. Biol.*, **19**, 205; 1965). Various attempts have been made to compare water losses from deciduous and coniferous stands by observational rather than theoretical methods, but most of these have shown negligible differences during the summer months (for example Zahner, *Forest Sci.*, **1**, 258; 1955); no reliable data are available for longer periods covering the winter season, when evergreens may continue to transpire and also to intercept more rainfall than deciduous trees. Comparative data between forest stands on different sites will always be somewhat suspect because of possible differences in the soil water storage capacities, hence long term studies on a single site where deciduous trees have been replaced by conifers seems to be the most hopeful approach to this problem.

The results of just such a study have now been published by Swank and Douglass (*Science*, **185**, 857; 1974). Their study area is in the Appalachian Mountains of the south-western region of North Carolina, originally bearing oak–hickory forest. Streamflow studies were carried out on selected watersheds within the area over a period of ten

years, which provided data sufficient for the prediction of stream flow under any given conditions of precipitation. In 1956 a 16 hectare watershed was clear-felled and planted with eastern white pine (*Pinus strobus*). For the next six years the streamflow was greater than that predicted for the forest stand, in the initial period by as much as 15%. Subsequently streamflow fell below predicted annual values and for the past 6 yr it has been between 15 and 20 cm (equivalent to 15–20%) below that expected from a hardwood stand on the same site.

Monthly streamflow values over the course of a year are particularly interesting because they show that between June and October the monthly streamflow is less than 1.5 cm below the expected value, whereas in November, December, April and May streamflow is over 2.0 cm below expected. Thus the additional losses of water from a conifer stand take place mainly in winter and spring; the possibility of this being the case had already been postulated by Penman (in *Forest Hydrology*, edit. by W. E. Sopper and H. W. Lull, Pergamon Press, Oxford, 373; 1967) on the basis of very little observational evidence.

The difference is likely to be caused by both the increased interception and the higher transpiration rates in the conifers in winter and spring. The leaf area index of hardwoods in winter is low (<1) in comparison with white pine (9.9), which indicates the much larger surface area with pine available for the interception of precipitation.

This provides one more good reason for caution in replacing hardwood forests with conifers, especially in water catchment sites.

Field effect seen in glassy semiconductors

from Andrew Holmes-Siedle

At the start of the solid-state age, Bardeen and colleagues attempted to modulate the conductivity of a slab of germanium by putting a metal plate near to the surface and applying a high electric field. Unexpectedly, the conductivity of the slab did not change and, because of this, the idea of the 'field-effect transistor' lay dormant for over ten years while the reasons for failure were explored. Meanwhile, in order to meet the urgent pressure for a working solid-state amplifier, the device makers (Sockley and colleagues) went on to develop the less elegant point-contact and diffused bipolar transistor principles.

The field-induced 'accumulation' or 'inversion' of current carriers in the germanium did not take place as predicted because immobile charge was generated in surface states. The sheet of charge 'screened' the interior of the semiconductor and prevented the required bending of energy bands. Nowadays, surface states in crystalline semiconductors are under fair control and the metal-oxide–silicon field-effect transistor is a standard component in computer circuits. It might now be said that we are at the start of the 'glassy solid state' age; it is perhaps a good omen that, despite a slow start, the field effect experiment can apparently be performed successfully in some glassy semiconductors. This is fortunate because the penetration of a semiconductor by a field provides a probe of band structure which is particularly appropriate for the glassy semiconductors.

Table 1 Estimated densities of localised states in chalcogenide glasses deduced from field-effect experiments

	Fritzsche	Egerton	Tick *et al.*
Typical composition	$As_{35}Te_{28}S_{22}Ge_{15}$	$Te_{50}Si_{12}Ge_{10}As_{10}$	$Te_2As\ Si$
Form	Evaporated	Evaporated	Melted and drawn
Bulk states ($eV^{-1}\,cm^{-3}$)	6×10^{19}	10^{19}–10^{20}	3×10^{17}– 2×10^{19}
Surface states ($eV^{-1}\,cm^{-2}$)	not estimated	2×10^{13}	2×10^{12}– 3×10^{16}

Spear and le Comber (*J. non-cryst. Solids*, **8–10**, 727; 1972) gained some information on localised states within the band gap of amorphous silicon, but H. Fritzsche failed to find any effect in the 'switching' materials, of chalcogenide composition, despite painstaking attempts (*Ann Rev. Mater. Sci.*, **2**, 697; 1972). R. F. Egerton (*Appl. Phys. Lett.*, **19**, 203; 1971) reported definite field effects which were, however, too small to be useful in determining the nature of the localised states. Now, by special methods of preparation, Tick and Watson of Corning Research and Development labs and Hindley of the University of Birmingham (*J. non-cryst. Solids*, **13**, 229–242; 1974) have prepared samples which have lower apparent densities of localised states and which thus give a higher response to the field effect. There is, therefore, some hope of using the field effect to determine the distribution with energy of the localised energy states in this very important group of materials.

The fabrication technique used was unusual. It is a fair assumption that the evaporated glass layers used by many investigators are more disordered than glasses cast and cooled from the melt. But a field-effect experiment on a sizeable cast block of glass would be hopelessly swamped by the conduction currents in the bulk of the sample. In order to make the bulk volume of the sample commensurate with the thin field-modulated region on the surface, Tick and co-workers used a thin filament of chalcogenide with an unusual form of surface passivation. The filament was produced by melting and drawing down a sample of chalcogenide powder enclosed in a special silica glass jacket. The result was a thin silica glass rod of diameter 7 mm with a very thin central filament of chalcogenide, of diameter only 0.01 mm. When this was sliced, one could gain contact to the ends of the filament with probes while the glass disk contributed support for an annular metal field plate as well as providing surface passivation and mechanical strength. For normal semiconductors and high fields, the surface space-charge regions are of the order of micrometres, so with these dimensions there is a chance that the field will influence a fair fraction of the filament volume.

In fact, negative fields of the order of 10^6 V cm^{-1} modulated the bulk conduction by about 5%, though greater fields produced little further effect; this could be termed a saturating dependence on field. Positive fields (metal positive) gave a much smaller effect. The simple analysis which best fits the saturating characteristic is that of a density distribution of localised states which is uniform with energy all across the band gap. The field dependence of current predicted for a sharply peaked distribution of localised states, such as that called for in the Davis–Mott model for chalcogenides (see for example Mott and Davis *Electronic Processes in Non-crystalline Materials*, Clarendon Press, Oxford, 1971) is of a very different form from that observed.

The model proposed also postulates a significant density of surface states. The figures for density are compared with the estimates made by Egerton and by Fritzsche in Table 1. The values given for certain samples in this latest work are seen to be considerably lower than in the earlier efforts—possibly a hopeful sign that we are learning better control of the preparation of glassy semiconductors.

These results, while not constituting a definitive disproof of the commonly accepted peaked energy distribution of the Davis–Mott model show that the field-effect method is a tool which, with perseverance, may provide definitive evidence on this question.

articles

Human reproduction and family planning: research strategies in developing countries

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The difficulties of providing family planning care on a world-wide scale have led to considerable emphasis on research in human reproduction. How much of this research should, and can, be carried out in developing countries raises many issues in research administration.

RESEARCH into human reproduction and family planning has expanded considerably in the past 15 years, and is now in progress in many institutions both in developed and developing countries. Rarely, however, has sufficient effort gone into formulating appropriate national policies for such research.

Developing countries raise some of the severest problems; frequently, esoteric research done in developed countries is repeated, while opportunities for local clinical investigations which are closer to the health needs of the countries are ignored. The nature of research training may be partly to blame; trainees may be exposed to a technique rather than being provided with an approach to research and a broader background in reproduction. The same incoherence is often found in the location of facilities: new institutes are created where expansion of existing ones would have been sounder and more economic. Funds are at times monopolised by small groups of scientists, and a contributory factor has been the multiplicity of funding sources, foreign ones often being the most important.

The urgency of this research and the relatively ready availability of funds means that policy issues require serious consideration. The World Health Organization recently convened a workshop for this purpose—research administrators from Argentina, India, Pakistan, Thailand, Turkey and the United States met to pool experiences. Their problems can be discussed under the following heads:

- Should research be done?
- How should priorities be determined?
- What administrative structure is necessary?

Should research be done?

Increasingly, policy makers are asking for clear orientation of research towards specific problems. Stephen Toulmin, in *Encounter*, has described this process as ". . . calling in the implied technological IOUs previously issued by the scientists". Policy makers also ask whether better use of existing knowledge and resources, or a simple increase in services would not better solve problems than would more research. Do methods of fertility control now available (whether by drugs, devices or surgical procedures) not meet the needs of the great majority of couples? Is not the need now to make these methods more widely available?

We believe there is still a need for research. The low initial acceptor rates and high discontinuation rates for present methods in almost all family planning programmes of developing countries are undoubtedly due in part to a lack of services; often, however, they are due to the wrong service approach. At the same time contraceptive technology still leaves room for improvement. Although more methods are now available, they remain relatively crude. At times they are associated with undesirable side effects, and they often fail to meet the needs posed by differences in culture, education, and service-structure. Moreover, many forms of contraception are still not available: a male pill, a post-coital pill, a once-a-month pill, a vaccine against pregnancy and so on.

Fertility regulation presents special problems for research: techniques require an assessment well beyond that required for other therapeutic modalities because of their extremely wide, long term and frequently unsupervised use. Further, expected users differ in genetic constitution and environment. In most cases return of fertility must be allowed and methods are usually self selected, not prescribed. Even conceding the need for more research, the role of developing countries in such research must still be clarified:

- For established methods, dosages appropriate for women of a high level of nutrition may not be applicable. Side effects of concern in the West may not manifest themselves, while new side effects may appear arising from disease and deficiency states in developing countries.
- For new methods, developing countries should be involved at an early stage of the research to determine acceptability. They must also be involved in testing at all stages of development so that safety and effectiveness data can be acquired rapidly for different populations.
- As far as services are concerned, the argument that family planning programmes can go forward on a common sense basis, without any service research, does not stand scrutiny. We have no precedent for providing health services on a continuing basis to most couples of reproductive age in a community. Sensible studies and small experiments are needed on the major components of family planning activities in a local context: information, education, and provision of clinical services. For instance, inappropriate presentation of educational material may spoil an otherwise well-designed programme. Local research is also essential in determining the right location of facilities, personnel, frequency and timing of clinics, schedules for follow-up care and provision of methods.

How should priorities be determined?

Data are frequently lacking on how to identify specific problems, how to rate their relative importance, how to determine their susceptibility to solution through research, what manpower and facilities are needed, what is the potential pay-off, and what is the likely duration and cost. On the other hand, fundamental questions of strategy have to be settled, such as the balance between goal-oriented and non-directed research, between research and research training, and between research in universities, government institutes and industry.

The usual guidelines for setting priorities in biomedical research are the levels of unresolved morbidity and mortality, and indices of general welfare. The same criteria hold here. Initially, broad problem areas must be identified and ranked in importance: pregnancy, labour, development, family planning and sterility. Then specific topics for study and research approaches must be ranked. For instance, should the emphasis be on development of new therapies or on service research?

The contribution of research to reducing morbidity and mortality has already been demonstrated in some fields, such as pregnancy and labour; beginnings have been made in research on the biology and health of the foetus and the foeto–maternal complex. This research has generally been in developed countries. Malnutrition and infectious diseases in developing countries are likely, however, to lead to great incidence of pathology and different forms of conditions associated with pregnancy, labour and foetal development.

In ranking family planning research, account must be taken of indices both of morbidity and mortality and of 'welfare'. Birth control prevents death and disease that result from unwanted pregnancy, short birth intervals, or illegally induced abortions, whilst the advantages of determining family size need little elaboration at the family level and will accord, in many communities, with population policies.

Problems of local importance undoubtedly have first priority in developing countries. The epidemiological, clinical, and service research required clearly cannot be carried out elsewhere. Amongst such problems are the effects of hormonal contraceptives in women infected with *Schistosoma haematobium*, foetal development in communities with a high incidence of thalassaemia and prenatal care in protein-deficient pregnant women.

More controversial are the priorities for problems that could be investigated in a developed country, for example, development of new contraceptives. Ideally, as we have said, any new drug should be investigated in developing and developed countries simultaneously, using a common protocol. This, however, demands highly specialised manpower and equipment. Should scarce resources be expended on the earlier stages of development, such as those that precede testing on human subjects, for instance, identification of reproductive processes susceptible to regulation, synthesis or isolation of suitable chemicals, screening for effectiveness and toxicological studies in animals?

Several arguments can be made for not automatically excluding from developing countries any aspect of research needed to resolve important problems. One is that it would make little sense and detract from the intellectual challenge if there were arbitrary cutoff points to research. Another is that the whole range of scientific expertise must be available in a developing country, since scientists and institutions in developed countries are often not interested in the problems of the developing country. An example is the lack of research on injectable contraceptives—a modality that meets many of the needs of developing countries. An artificial limitation would further deprive the scientific community of talent and would preclude scientists in these countries from pursuing freely their choice. Finally, university teaching is improved by the presence of an active research programme.

Should public funds support research that is left entirely to the choice of the individual? A comparison of non-directed with directed research is difficult to make, and the level of funding for non-directed research cannot yet be determined on the basis of general principles.

Feasibility of research on priority problems

A problem may be important and it may be seen to be amenable to solution through research, but it will have to be ranked in terms of the manpower and facilities that it would tie up. Work can sometimes start immediately on a problem not of the highest priority, while a more important one must await the necessary resources. A 10–20 yr plan, regularly reviewed, is needed.

A representative ranking of research areas in many developing countries could take the following form. First, the health rationale for family planning (for example, studies of effects on health of different patterns of family formation, identification of women particularly requiring family planning care because of their health status); second, clinical trials of contraceptives at the community level; third, the effects of widely-used contraceptives in the presence of conditions such as malnutrition, diseases, use of other drugs; fourth, the acceptability and service implications of different methods of contraception; fifth, sterility, and, sixth, the in-depth, pharmacological studies in humans of new contraceptives.

Some developing countries may be able to start rapidly on the first item as they may already have manpower in the required disciplines such as obstetrics and gynaecology, maternal and child health, sociology, biostatistics. Specialised technicians may need to be trained for field work, computer analysis, and clinical laboratory measurements. This might take about three years. If centred around one or two good investigators, a research nucleus would have been created which could then, if required, move into research projects in almost all of the priority areas.

In contrast, item six demands manpower and facilities unlikely to be present in many countries. Several years may be needed to train specialists with skills in steroid assays, peptide chemistry, radioisotope use, pharmacology, metabolic studies and so on. Technicians are also essential as is the availability of specialised clinical facilities.

One issue that repeatedly comes up is the proportion of funds to be spent on defined projects as opposed to longer-term more comprehensive 'institution-building' ones. Assurance of financial support over a number of years is of importance to all investigators, but particularly so in the case of institution-building.

A shortage of qualified manpower may make it difficult to bring together experts, other than the applicants themselves, to review research proposals for scientific merit, so foreign assistance may be necessary. The low level of literacy in a population may pose problems in ensuring that 'informed consent' has been obtained.

Should research training be restricted, for instance, to clinical trial methodology, or widely based in clinical pharmacology? The narrow approach quickly produces an investigator able to apply himself to an important problem; the more broadly educated scientist is potentially more versatile. During his training, however, he may find his interests shifting away from clinical trials. Western research training for scientists from developing countries has been problematical: we may mention the 'brain drain' and the lack of orientation of the research training either to problems or conditions the scientist will encounter on his return home. Failure to provide a research position for the

investigator on completion of his training, to ensure attractive career opportunities regularly leads to loss of talent to the developed countries; some countries are now trying to remedy these shortcomings.

Keeping up with rapid advances presents a technical and financial problem. Airmail subscriptions to journals, access to computer retrieval of bibliographies, participation in international meetings go some way to help. Less attention has been given to ensuring that research results rapidly reach policy-makers, administrators and service personnel in a form appropriate to their needs.

What administrative structure is necessary?

The number of agencies with interests in research in this field is probably greater than in most other areas of biomedical research. The concerns of such bodies as the Ministry of Health; the national Family Planning Authority; the Ministry of Education; the Medical Research Council (MRC); the Council for Science and Technology; the government offices responsible for the national drug industry; the national drug regulatory agency and so on, may be difficult to unite in a common policy. It may be even harder to bring in the private drug industry whose orientation is primarily commercial. Yet another factor is the aggressive attitude sometimes taken to family planning, including its research aspects, by foreign aid assistance agencies, whose priorities may not necessarily coincide with national ones.

The national MRC sometimes offers a plausible focus: it has experience in formulating biomedical research policy, could place research in reproduction and family planning within the overall context of the country's biomedical research programme, and could use its existing mechanisms for administration. In many developing countries, however, there may not be an MRC capable of assuming these responsibilities; a ready focus may then exist in the Ministry of Education in whose institutions much of the research will be carried out anyway. Another approach creates a body within the national family planning authority for the administration of research. This should ensure a research policy directed to the problems encountered in the family planning programme and ensure rapid transmission of research results to field workers; unfortunately, such bodies may have little tradition of research, encounter resistance from older established ministries, and restrict themselves to a narrow and short term view of research.

External aid has often been forthcoming, but on occasions not so much to support research efforts as to influence family planning policies, or even to gain access to subjects to test drugs under less stringent regulatory conditions than those prevailing in the industrialised countries. The magnitude of the funds offered has at times led to this assistance being accepted in spite of the distortion it brought to nationally selected priorities. Some countries now place rigid embargos on accepting assistance from any other than multilateral sources.

The most neglected use of foreign assistance has been in the areas of policy and research organisation. Few donors have wished to support activities that have little visibility and no quick returns, and few recipients have insisted on using research funds for this preliminary ground work.

Compositional variation in recent Icelandic tholeiites and the Kverkfjöll hot spot

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Geochemical studies of recent igneous rocks from Iceland lend support to the 'hot spot' theory. There is a geographical variation in basaltic composition between Iceland and the submerged parts of the Reykjanes Ridge and Mid-Atlantic Ridge, which suggests that the single-source model for basalts of this region may be correct.

THE puristic mantle plume hypothesis[1-3] holds that all ocean ridge volcanism is fed by the plumes. The plate systems drift passively across the Earth's surface relative to the plumes, as shown by the universal symmetry of magnetic anomaly patterns about the ocean ridges. Thus, the position of the ridges is not fixed by subcrustal agencies such as convection cells in the mantle. Outside the mantle plumes there are no heat sources other than the plume overflows. All the heat flow in the ocean bottom can be accounted for by the loss of heat from the cooling plates as they drift away from the ridges[4].

The single-source theory has been challenged by Schilling[5] on geochemical grounds, particularly with reference to Sr-isotope variation[6]. We propose to show that geochemical variations in Iceland and the North Atlantic may be consistent with the one-source model, both as regards petrochemistry and isotope geochemistry.

We have collected and analysed 182 samples that are considered to cover volcanic activity in the tholeiitic rift zones of Iceland[7] during the last 10^5 yr. The geological formations represented are the 'grey dolerites' from the last interglacial, pillow lavas and breccias of the subglacial moberg (palagonite) formation, and postglacial lava flows. The volumes of the individual lavas vary greatly: monogenetic shield volcanoes range from 1–15 km^3, whereas lavas produced on fissures and isolated scoria cones are much smaller—generally less than 1 km^3.

The samples have been divided into three groups on a geographical basis (Fig. 1) representing the western rift zone (WRZ, 126 samples), the northern part of the eastern volcanic zone[8] (NEZ, 26 samples), and the Kverkfjöll area (30 samples), a relatively small area in the vicinity of the Kverkfjöll volcanic complex in central Iceland (K. Albertson, unpublished).

The Kverkfjöll area is the hot spot of the North Atlantic. Away from that area the maximum content of potassium in basalts on the volcanic rift zones decreases continuously with distance (Fig. 2). The minimum K_2O content remains constant, at about 0.05–0.1%, in the Kverkfjöll area and probably along the entire adjoining ridges. A pro-

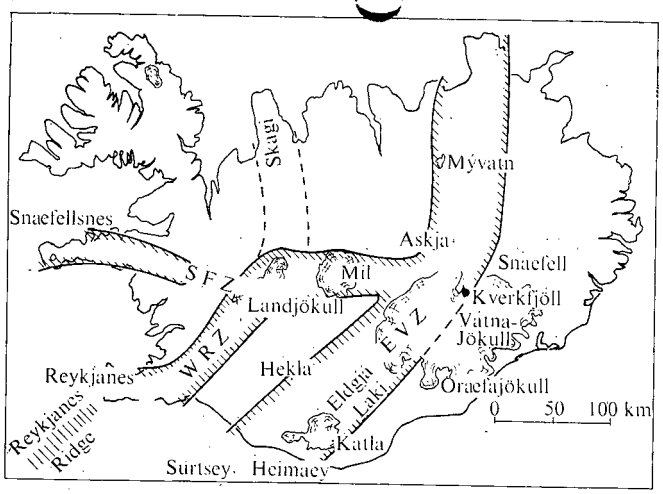

Fig. 1 Neovolcanic areas in Iceland. The Snaefellsnes and the southern part of the eastern zones are alkaline in character, the others tholeiitic. WRZ, Western Rift Zone; EVZ, Eastern Volcanic Zone; SFZ, Snaefellsnes Fracture Zone.

nounced positive correlation exists between K_2O, TiO_2 and P_2O_5 whereas K_2O and Rb show insignificant covariation. In central Iceland basalts with a high Rb content exist side by side with basalts highly depleted in Rb. Away from the Kverkfjöll area the basalts show gradual depletion of Rb towards the ocean ridges together with increasingly narrower spreads in K_2O values. At a distance of 500–600 km from central Iceland the K_2O content of the basalts reaches a value of 0.1%, with very little spread around that figure.

The samples from the NEZ have been discussed in detail by Sigvaldason[8]. A model was proposed to explain the large compositional spectrum in Icelandic basalts as compared with the narrow range observed in the bulk of ocean ridge basalts. The analyses presented here corroborate those results. In the model it is assumed that the Icelandic mantle plume feeds all volcanism in the North Atlantic. The thermal gradient within the plume centre increases continuously to great depths in the mantle, providing a thermal environment for partial melting over a large depth range. Conversely, as the plume overflow spreads below the crust along the volcanic rift zones in Iceland and the adjoining ridges, the thermal gradient goes through a temperature maximum at which partial melting occurs. With increasing distance from the plume centre, the thermal maximum becomes better defined at a depth which happens to be appropriate for the formation of ocean tholeiites. As that represents relatively low-pressure partial melting, all chemical types formed by partial melting at higher pressure gradually disappear (Fig. 2). Thus, two processes are in evidence: partial melting at varying depths, and progressive depletion of dispersed elements at the source.

The Icelandic tholeiites span almost the entire spectrum of observed compositions of oceanic tholeiite. To illustrate this is a plot of MgO against iron (Fig. 3) compares the Icelandic tholeiites to analyses of oceanic tholeiites[9–11]. In spite of this great compositional span, however, the bulk of the Icelandic analyses tends to fall on the iron-rich side of the oceanic analyses. This is in keeping with the fact that, as regards volume and frequency, Icelandic tholeiites are distinguished by high alkalis and iron magnesium ratios[12] relative to oceanic basalts. The Juan de Fuca[11] analyses resemble the Icelandic suite most (Fig. 3), which is in accord with the idea that the Juan de Fuca ridge is the site of a mantle plume[2].

Within the Icelandic group the enrichment of iron and potassium, together with other related chemical variations,

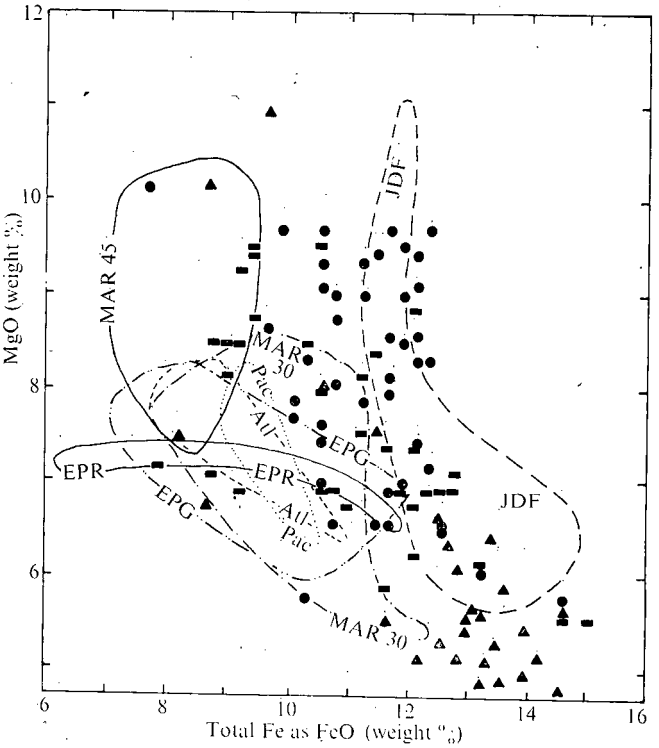

Fig. 3 MgO plotted against total iron in the samples, showing the relatively iron rich nature of Icelandic tholeiites. The range of values observed in oceanic tholeiites is shown for comparison. Atl, Atlantic Ocean[9]; Pac, Pacific Ocean[9], EPR, East Pacific Rise[11]; EPG, Gorda ridge (41° N)[11]; MAR 45°, Mid-Atlantic Ridge (45° N)[11]; MAR 30°, Mid-Atlantic Ridge (30°N)[11]; JDF, Juan de Fuca Ridge (44°N)[11]; ▲, Kverkfjöll Area; ■, North-eastern Zone; ●, Western Rift Zone.

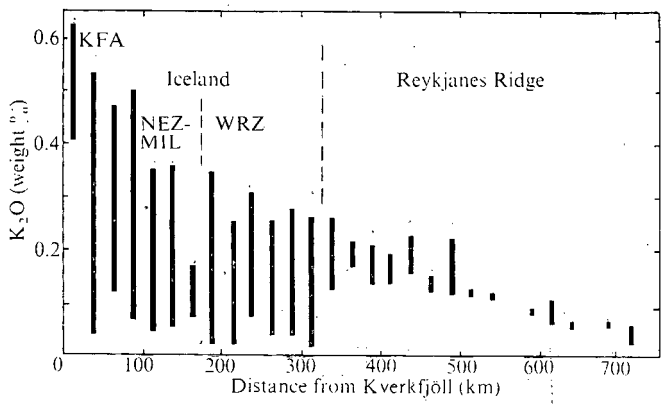

Fig. 2 Range of K_2O values plotted against linear distance from Kverfjöll, central Iceland. Each column represents the range obtained in a 25 km sector. KFA, Kverfjöll area; NEZ, northern part of Eastern Volcanic Zone; MIL, Mid Iceland; WRZ, Western Rift Zone; (see Fig. 1).

seems to bear an inverse relationship to lava volume[8]. This effect could be attributed to fractional crystallisation[11]. The Ti concentration, however, which has been shown to be sensitive to pressure[13], shows close positive covariation with K, and could be explained equally well by a degree of partial melting (compare with Fig. 2). The basaltic liquids may have formed over a relatively large depth range in the mantle. Frequent eruptions of small batches of lavas which have undergone only a low degree of partial melting, and which have been derived from a considerable depth, result in large total volumes of high alkali, high iron/magnesium tholeiites, relative to low potassium tholeiites which are produced at shallower depths by a higher degree

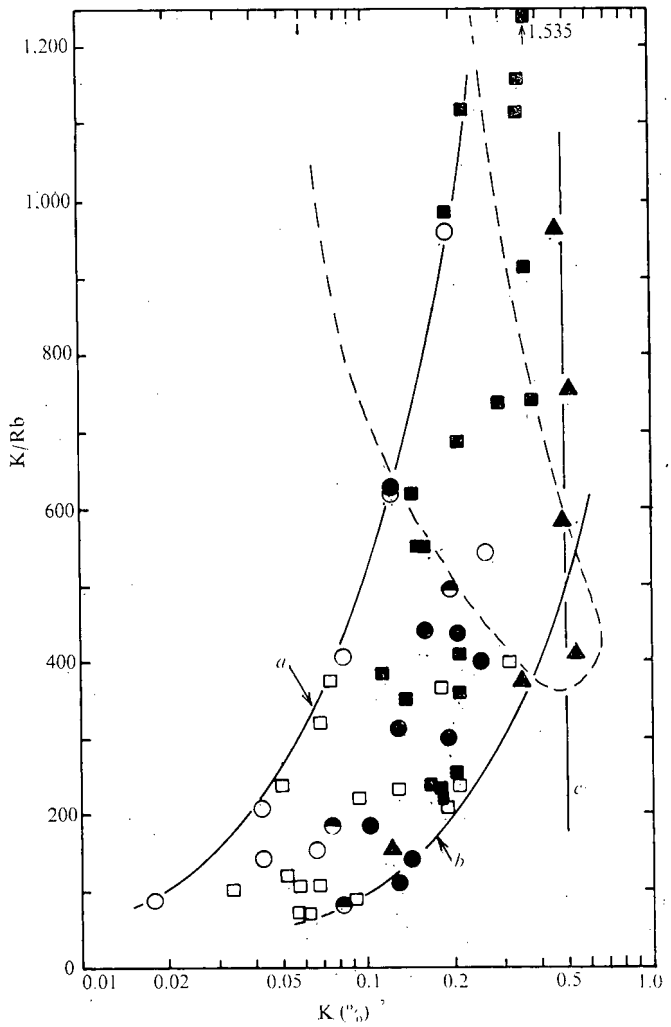

Fig. 4 The K/Rb ratio plotted against log K. Lower part of the field of oceanic tholeiites[11,16] enclosed by dashed line. *a*, Rb=2 p.p.m.; *b*, Rb=10 p.p.m.; *c*, K=0.5%; □, NEZ shield volcanoes and table mountains; ○, WRZ shield volcanoes and table mountains; ■, NEZ fissures and minor eruptions; ●, WRZ fissures and minor eruptions; △, KFA fissures and minor eruptions. The Rb values were obtained by XRF analysis. Although this has a distinct disadvantage at low Rb values this was overcome partially by adding a fixed amount of 20 p.p.m. Rb to the samples. The precision is considered to be better than ± 1 p.p.m.

of partial melting. The volume relationships between products of partial melting of high and low degree in Iceland are, therefore, entirely different from those of the ocean ridges. Along the Reykjanes ridge, in a direction away from Iceland, the low degree partial melting products disappear, giving an impression of an increase in the degree of partial melting at the same time as volcanic productivity decreases. This seems to explain adequately the apparent decrease in light rare earth elements south of Iceland[5].

Figure 4 shows the K/Rb ratio plotted against K for some of the samples, with the field of ocean ridge basalts[11,16] shown for comparison.

Several features of the diagram are worthy of discussion. The K/Rb values are generally lower than previously observed for tholeiites[11]. Analyses of plagioclase separates from porphyritic basalts show K/Rb ratios in the range 50–300. There is no consistent relationship between the K/Rb ratios of phenocrysts and groundmass. Furthermore, REE analyses of Icelandic tholeiites[14,15] do not show Eu anomalies, suggesting that there has been no plagioclase differentation. These low ratios, therefore, may be derived from the mantle and could indicate a higher content of Rb (phlogopite for example) in the plume than has been assumed previously[17].

The K/Rb–K field is defined by three curves (Fig. 4). its low K side is bounded by the curve Rb=2 p.p.m., whereas for the high K side, at K/Rb ratios below 400, the curve Rb=10 applies, and for higher values the line K=0.5% (Fig. 4). The values for the shield volcanoes tend to fall on the diagram to the left of those of other type of volcanoes. As a group the shield volcanoes show the lowest K and Rb values in Iceland even if the K content may be higher, and the Rb content is much higher, than in the depleted ocean ridge basalts. The voluminous shield eruptions occurred in the specific volcano-tectonic environment found during the isostatic rebound of Iceland immediately after the Pleistocene glaciation. The prevailing conditions of pressure and temperature were suddenly disturbed towards lower pressures, resulting in the melting of large batches of mantle material that had already gone through an earlier stage of melting. They are the most depleted basalts in Iceland.

Schilling[5], chiefly on the basis of Sr isotopes[6], has advocated two primary magma sources in the North Atlantic. Magmas derived from the Icelandic plume have high (~ 0.7032) $^{87}Sr/^{88}Sr$ ratios[6,15,18], show an undepleted REE pattern, and have generally high contents of K, Ti, P, and high Fe/Mg ratios. The oceanic source yields lavas with a much more depleted chemistry and lower (0.7026–0.7028) Sr isotope ratios[6]. The gradient in composition, from Iceland along the ridge, results, according to that theory, from the mixing of the two magmas. The observed variation in Sr isotopes can possibly be accounted for in terms of the single source model. At the depth at which magma segregation is supposed to take place the upper mantle consists of olivine, two pyroxenes, plagioclase and minor phases, including phlogopite[19] and amphibole[20,21]. The K and Rb enrichment of the basalts in central Iceland results from the important contribution of phlogopite to the early partial melts of the plume. Phlogopite contains about 7% K, 250 p.p.m. Rb, and 18 p.p.m. Sr (ref. 17). The other components in the upper mantle contain between 1 p.p.m. and 0.1 p.p.m. Rb. The high Rb content of the phlogopite results in an increased $^{87}Sr/^{86}Sr$ ratio relative to the rest of the mantle. Using the values of Rb and Sr already given for phlogopite the $^{87}Sr/^{86}Sr$ ratio would increase by 7×10^{-5} every million years. Conversely, the highest Rb/Sr ratios observed in Iceland tholeiites increase by 2×10^{-6} every million years, whereas the Sr isotopic ratio of the depleted ocean tholeiites has very little capacity for change with time and probably reflects that of the bulk of the upper mantle.

The implication of possible isotope disequilibrium between mineral phases in the upper mantle may be far reaching as regards the interpretation of isotopes in partial melts. As long as a given mineral assemblage remains stable, however, each mineral may develop its own isotope ratio—which is the basis for the isochron method of radiometric dating[22]. Unless recrystallisation occurs there seems little impetus for isotope re-equilibration between phlogopite and the mantle phases with a low Sr content. In general, because Sr is produced in phlogopite by radioactive decay it should tend to migrate one way only—away from the phlogopite.

There is, as yet, insufficient documentation of the relationship between precisely measured Sr isotope ratios and the petrochemistry of Icelandic rocks. Preliminary work[6,15] indicates, however, that, in general, rocks from the alkalic provinces in Iceland possess slightly higher Sr isotope ratios than the tholeiites. That is consistent with their supposed nature as lower degree partial melts[19].

On the basis of the available data we therefore conclude that there is no well established geochemical evidence to

contradict the single-source model for the North Atlantic. There is a continuous gradational variation between basalts erupted in Iceland and basalts on the submerged part of the Reykjanes and Mid-Atlantic Ridges. This compositional gradient probably results from the combination of a changing environment of pressure and temperature in an upwelling and radially spreading mantle plume system, and from a progressive depletion in the low temperature melting fraction at the source.

Note added in proof: Unknown to us at the time of writing, similar ideas on isotope disequilibrium in the mantle have been published[23].

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Climatic significance of deuterium abundance in growth rings of *Picea*

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Deuterium variations in growth rings of a Picea *from southern Germany are essentially a function of annual air temperature. Short term isotope variations are partly influenced by changes of relative humidity, but it is the long term annual temperature fluctuations which are mainly responsible for the deuterium variations in the rings. Long term deuterium variations in* Picea *record climatic changes of the past and also reflect the deuterium content of the annual precipitation.*

NATURAL, stable isotopes are used more and more frequently in the field of palaeoclimatic research. Apart from ^{18}O thermometry[1,2] on sea cores, measurement of the deuterium content of plants opens new aspects for palaeoclimatic investigations.

The basic idea of the deuterium method is that the natural deuterium content of chemically bound hydrogen in plants is correlated with that in precipitation[3]. As the deuterium content of precipitation in middle and high latitudes depends mainly on the climate and decreases with annual mean temperature[4], the deuterium content in plants is closely related to climate[5], and tree rings should therefore bear information on past climates.

Sample preparation and mass spectrometric deuterium determinations have already been described[3]. The overall measuring accuracy is $\pm 2‰$ and the results are reported in per mille deviation from Standard Mean Ocean Water (SMOW)[6].

During evapotranspiration of the leaves, complicated isotopic interactions occur between atmospheric vapour and water which is replenished from the soil. The hydrogen component of the water is transformed by photochemical processes into woody matter and so evapotranspiration is a process which is important in the development of the deuterium content of plant material.

Isotopic equilibria in these interactions can be described mathematically[7]:

$$\delta_l = \delta_s(1-h) + h\delta_v + \varepsilon_p + \varepsilon_k(1-h), \quad (1)$$

where δ_l is the deuterium content of the leaf sap, δ_s that of soil water, and δ_v that of atmospheric vapour. ε_p is the equilibrium separation factor which increases with decreasing temperature, ε_k is the kinetic separation factor, and h the relative humidity. This formula describes the isotopic processes during evapotranspiration in leaves to a good degree of approximation[8].

The average deuterium concentration in leaves from a *Picea* was calculated for the period 1881–1970. For the calculations I used meteorological data from the Hohenpeissenberg Observatory (unpublished information and ref. 9), which is 16 km east of Sachsenried, the place where the tree grew. Measurements of the deuterium content of precipitation and ground water of this area indicated that $\delta_s \approx 70‰$. Because atmospheric vapour in Central Europe, contains on average, 75‰ less deuterium than does precipitation: $\delta_v \approx \delta_s - 75$ (ref. 10). ε_p is temperature dependent and is +93‰ at 15° C (ref. 11), and ε_k (= 16‰) is independent of temperature. The relative humidity in the area around Sachsenried is about 75‰, and

Table 1 Correlation coefficients between the deuterium content of *Picea* and meteorological and other parameters

	1785–1910	1910–1970	1785–1970	1785–1970
	5-yr means			10–20-yr means
$r_2\sigma$	0.4	0.6	0.33	0.54
Summer temperature	0.035	0.52	0.04	0.41
Annual temperature	0.51	0.74	0.47	0.83
Humidity	—	0.47*	0.056*	0.34†
Leaf sap	—	0.57	0.43*	—
δ_{18} ice	—	—	—	0.61

* $r_2\sigma = 0.49$, † $r_2\sigma = 0.7$ (beginning in 1881).

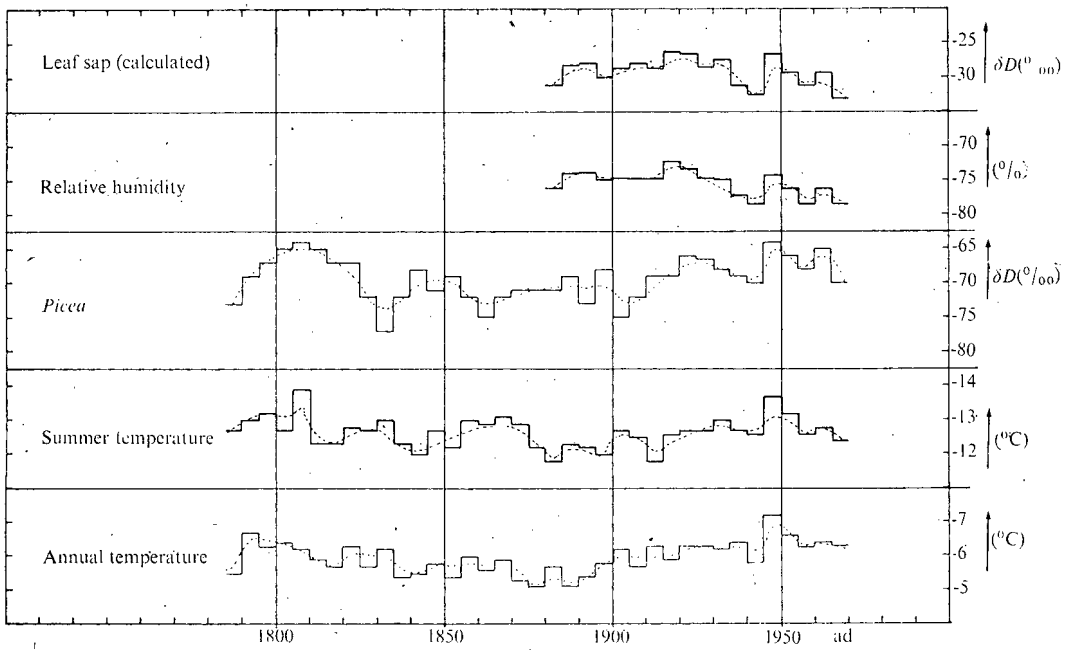

Fig. 1 Comparison between summer temperature (mean May–September), annual mean temperature, deuterium content of *Picea*, relative humidity (mean May–September) and the calculated deuterium content of the leaf sap all in the 5-yr means. Dashed lines indicate the trends in the histograms.

so equation (1) shows that the influence of the atmospheric vapour is more significant than the influence of the water which the plants take up.

Implications of a deuterium content

From equation (1) it follows that the deuterium content should depend essentially upon the temperature and relative humidity during the growing season. In order to test this conclusion the deuterium content of growth rings of a *Picea* from Sachsenried (46° 50′N, 10° 50′E), with an age of about 200 yr was determined. For the period 1910–1970 each ring was analysed twice for deuterium to diminish any errors which may have arisen from the analysis of an incomplete ring. Growth rings formed between 1785–1910 were analysed. In each case a block of rings formed in five consecutive years was analysed and care was taken to burn the same amount of wood from each block, so that a weighted mean was not obtained.

The deuterium content of each block has been compared with the summer temperature (mean May–September), the annual mean temperature, the relative humidity (mean May–September) (Fig. 1), and the calculated deuterium concentration in the sap of leaves (for the same summer period) using equation (1). In order to be able to compare the results for the various possible combinations in a statistically meaningful manner the correlation coefficients for the various periods with different measuring accuracies have been calculated (Table 1). The maximum error of the correlation coefficient for the 2σ limit is given as $r_2\sigma$ in Table 1.

In the 5-yr mean, there is a reasonable correlation between the deuterium content and the summer temperature ($r = 0.52$; $r_2\sigma = 0.6$) in contrast to the poor correlation with mean annual values. In particular, the deuterium trend is distinctly indicated by the trend of the temperature curve. A perfect correlation cannot be expected in any case, because the meteorological parameters are averaged continuously over five months—that is not the case for the deuterium content of wood. On overcast days there is hardly any assimilation because of insufficient intensity of solar radiation. Furthermore, the humidity still shows some fluctuations in the 5-yr mean, so some degree of influence of the humidity on the deuterium content is evident. On the long term mean the humidity variations level off and the trend of the deuterium curve follows more perfectly that of the temperature curve (Fig. 1).

The measuring accuracy is best for the period 1910–1970,

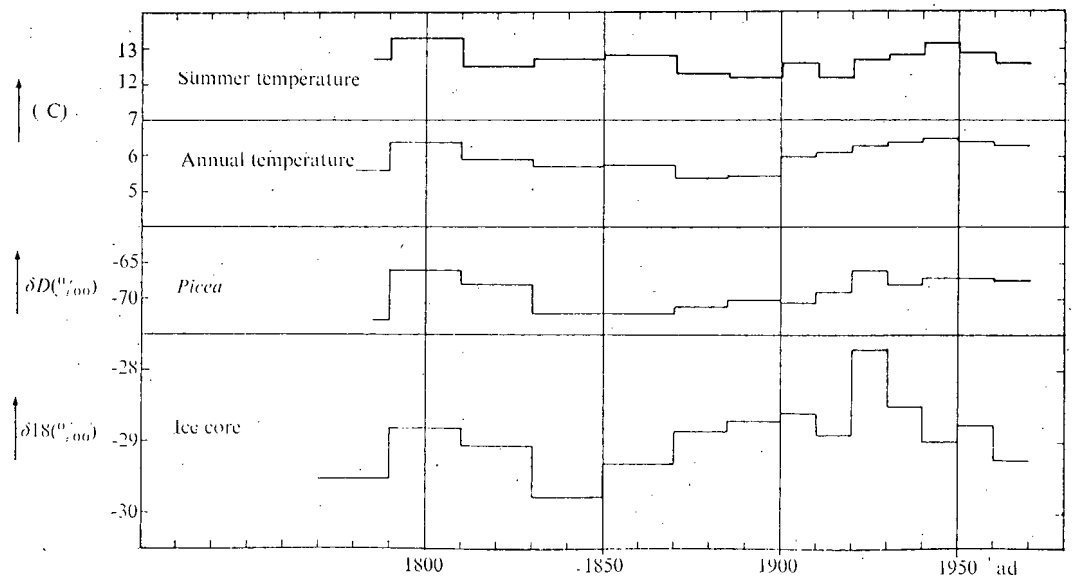

Fig. 2 Comparison between the ^{18}O content of an ice core from Camp Century on Greenland and the deuterium content of the growth rings of a *Picea* from Sachsenried. The upper graphs show the summer and the annual mean temperatures from the Hohenpeissenberg, Meteorological Observatory near Sachsenried.

because the average of 10 single measurements is used. In the period 1785–1910 the accuracy is poorer because only two single analyses are averaged (Table 1) and the time scale for the older year rings becomes increasingly inaccurate because some rings are missing.

The deuterium variation of the leaf sap is smaller than that of the growth rings. Presumably, that is because the calculation is based on a constant correlation between the deuterium content of atmospheric vapour and the atmospheric temperature (3‰ $^{\circ}C^{-1}$) for Central Europe. Such a relationship, however, is an idealisation of the natural conditions, because at any one temperature, depending on the meteorological conditions, substantial variations in the deuterium content of atmospheric vapour can still occur.

The growing period comprises hardly half a year, and so it must be considered whether the climatic processes during that period really reflect, to any degree, the general climatic trend of the entire year. A comparison between summer and annual temperatures shows that similar trends do, in fact, occur. The low temperatures of the 19th century, which caused a considerable advance of the mountain glaciers, in temperature latitudes, are indicated clearly. In addition to that, a comparison of the correlation coefficients for summer and annual temperatures (Table 1—5-yr means) shows that the annual temperature correlates much better with the deuterium content of the growth rings ($r = 0.47$; $r_2\sigma = 0.33$) than does the summer temperature ($r = 0.04$; $r_2\sigma = 0.33$). That observation is further supported by the comparison in Fig. 2. In the lower graphs the deuterium content of *Picea* is compared with the ^{18}O result from an ice core from Camp Century on the Greenland ice sheet[12]. There is a good relationship, although the ice reflects the isotopic composition of the annual precipitation ($r = 0.61$; $r_2\sigma = 0.54$). On the long term mean the relationship between the deuterium content again seems better with the annual temperature ($r = 0.83$; $r_2\sigma = 0.54$) than with the summer temperature ($r = 0.41$; $r_2\sigma = 0.54$). I therefore conclude that the deuterium concentration in the atmospheric vapour is a function of the climatic history of the whole year and influences the deuterium content of the leaf sap so that, even though only a relatively short growth period is measured, the results reflect throughout a whole year. That is important for all palaeoclimatic investigations which use deuterium variations in biogenic material. Finally, as two localities thousands of kilometres apart show similar isotopic trends, it follows that essentially global climatic changes must be responsible for the deuterium variations in *Picea*.

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Properties of hybrids between *Salmonella* phage P22 and coliphage λ

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The temperate bacteriophages P22 of Salmonella typhimurium *and* λ *of* Escherichia coli *form viable hybrids in which the immunity, early control and DNA replication genes of P22 are substituted for the analogous* λ *genes. The specificity of the early control and DNA replication genes differs between P22 and* λ*; the immunity of the hybrid is identical with that of the lambdoid coliphage 21. Implications for the evolution of viruses are discussed.*

THE temperate bacteriophages P22 of *Salmonella typhimurium* and λ of *Escherichia coli* are very similar in functional organisation[1,2]. In particular, the regions concerned with control, recombination, integration, DNA replication and lysis functions of both phages are arranged in the same order on their genetic maps (Fig. 1). The phages share DNA sequence homology, as measured by DNA–DNA hybridisation[3], most of which occurs in the right arm of λ DNA[4], where the functions listed above are located. These similarities are counterbalanced by profound differences between the two phages. (1) P22 DNA is circularly permuted and terminally repetitious, whereas λ DNA is not permuted and has single-stranded cohesive ends[5,6]; (2) The P22 genetic map is circular, whereas the map of λ is linear[7,8]; (3) P22 particles have only a short baseplate structure whereas λ has an elongated tail; (4) P22 adsorbs only to *Salmonella typhimurium* whereas λ adsorbs only to *E. coli*; (5) Control of lysogeny differs: P22 has two regions essential for immunity and repression whereas λ has but one[11,12]; (6) P22 lysogens exclude superinfecting phages in several ways beyond the immunity and repression system whereas λ lysogens display solely the immunity provided by the single repressor[13,14].

These considerations led us to inquire whether hybrid phages containing DNA from both P22 and λ could be constructed, and, if so, which blocks of P22 genes were interchangeable with analogous blocks from λ. We show here that P22 DNA can be substituted in the right arm of λ to yield hybrid phages with distinctive properties. The construction of P22/λ hybrid phages was accomplished independently by a different method by Gemski *et al.*[15]. Studies

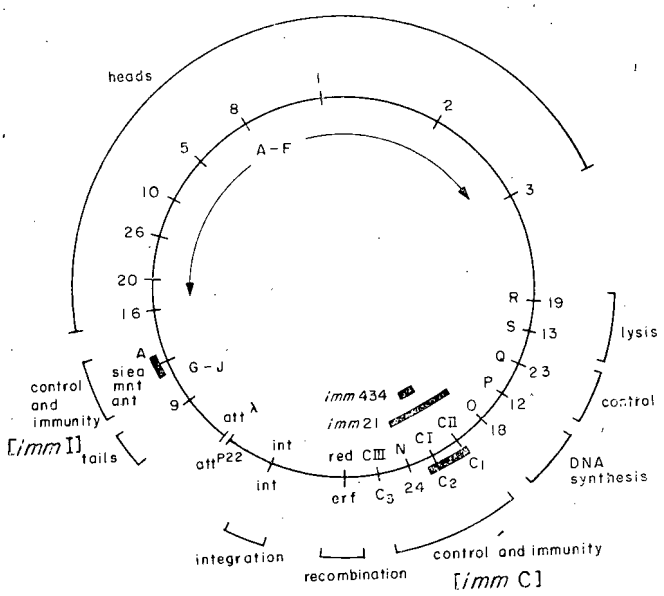


Fig. 1 Comparison of the genetic maps of P22 and λ. Inside the map are the λ gene designations; outside are the P22 gene designations. One-to-one correspondence means great similiarity in mutant phenotype. The heavy bars outside indicate *imm*I and *imm*C, the heavy bars inside indicate the extent of non-homology between λ and λ*imm*21 and λ*imm*434. The map is redrawn from Botstein *et al.*[1].

with these hybrid phages indicate that P22 and the *E. coli* lambdoid phage 21 (ref. 16) have repressors and operators with apparently identical specificity, indicating common ancestry; on the other hand, the DNA replication and early gene control systems of P22 and the lambdoid phages are analogous but have significantly different properties.

The cross

Phage P22 does not adsorb to *E. coli* and phage λ does not adsorb to *S. typhimurium*. To get both phage DNAs into the same cytoplasm so that they might recombine, we took advantage of the ability of *E. coli* episomes to be transferred between the two species of bacteria. Roth and Hoppe[17] showed that the F′*lac*$^+$*pro*$^+$ episome of *E. coli* carries a prophage attachment site for phage P22. Consequently, P22 was inserted into this episome by infection of an F′*lac*$^+$*pro*$^+$ *Salmonella* strain which was deleted for the P22 prophage attachment site on the chromosome. The P22 carried on this episome has a temperature-sensitive repressor (*c*2 gene product); hence, the prophage is induced at high temperature in *Salmonella* strains. The F′*lac*$^+$*pro*$^+$*str*^{s} (P22*c*2-*ts*) *Salmonella* strain was mated with an *E. coli lac*$^-$*str*^{r} strain, and *E. coli lac*$^+$*str*^{r} recombinants selected. Most of these recombinants were temperature-sensitive for growth and produced 20–50 P22 phage per cell at high temperature. Therefore, it appears that all host components required for P22 growth are present in wild type *E. coli*. In contrast, it was found that λ$^+$ will not grow under similar circumstances in *S. typhimurium*[18].

The hybrid phages described here are derived from an *E. coli* lysogen-carrying P22*c*2*ts*30 prophage; when this lysogenic strain was infected with a marked λ (λ *c*I60) and the culture was shifted to 41° C, a small yield of λ and P22 (about 20 of each) was produced. Among the progeny were recombinants (about 0.01%) with the host range of λ (that is, adsorption to *E. coli*), but which had acquired a new immunity (that is, growth in *E. coli* lysogenic for λ). In a parallel infection, nonlysogenic but otherwise identical F′*lac*$^+$*pro*$^+$ *E. coli* strain was infected with λ *c*I60 at 41° C; no phages able to grow on *E. coli* lysogenic for λ were obtained.

Structure of λ*imm*P22

(a) The immunity region derives from P22. Since the hybrid phages were selected for their ability to grow on a λ lysogen of *E. coli*, it was expected that the hybrids had a new immunity, presumably from the P22 prophage. This idea was confirmed by several observations. (1) The hybrid phages all have a temperature-sensitive clear plaque morphology, unlike that of the λ parent, but like that of the P22 prophage. As expected if the *ts*-clear mutation affects the P22 *c*2 gene, *E. coli* lysogens carrying these hybrids as prophages are induced when shifted to 41° C. (2) λ phages grow on the *E. coli* strain lysogenic for P22. The hybrid phages do not grow on these lysogens, indicating that they have immunity of P22. The hybrid phages grow normally on the F′*lac*$^+$*pro*$^+$ *E. coli* strain which does not carry a P22 prophage (Table 3) (3) In other experiments, hybrids were constructed between λ and P22 prophages which carry *amber* or *ts* mutations in the genes near the *c*2 gene; in these cases, the hybrid phages inherit the *amber* or *ts* phenotype (S. Hilliker, unpublished work).

These bybrid phages—obtained from the cross of λ*c*I60 with P22*c*2*ts*30—are called λ*imm*P22 by analogy with λ*imm*434 and λ*imm*21, λ hybrids with the immunity specificity of the lambdoid phages 434 and 21 (refs 19 and 20).

(b) Tail genes derive from λ. Since the hybrid phages can grow on *E. coli* strains, the genes determining host range must be inherited from the λ parent. As anticipated, the hybrid phages are as sensitive as λ wild type to antibodies raised against λ wild type phage particles. Furthermore, mutants of *E. coli* lacking the λ receptors for adsorbtion are also resistant to the λ*imm*P22 hybrids (data not shown). λ*imm*P22 hybrids, therefore, have tail genes from λ.

(*c*) Prophage insertion specificity derives from λ. The hybrid phages can integrate into an F′*gal att*λ episome and be tranferred into another strain by conjugation. λ*imm*P22 hybrids, therefore, have the insertion specificity (attachment site and *int* gene) of λ (S. Hilliker, unpublished work).

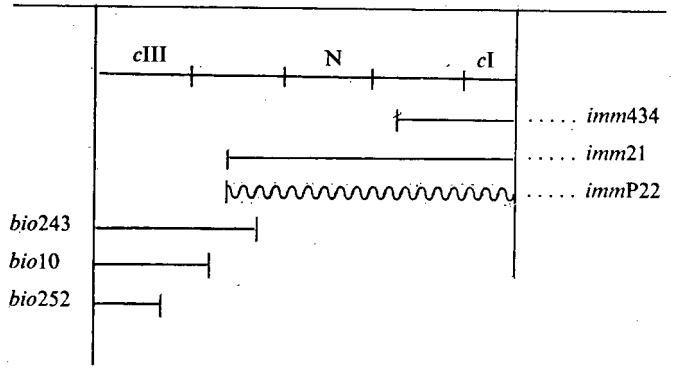
(*d*) The source of other genes in the hybrids is as follows. Marker rescue tests were done by plating λ *amber* mutants on *su*$^-$ (nonpermissive) lysogens of λ*imm*P22 hybrids. For comparison, phage were also plated on lysogens of λ*imm*434 (known to substitute none of the essential λ genes tested) and λ*imm*21 (known to substitute only gene *N*)[21]. Recombinants with immunity of λ and wild type gene function

Table 1 Efficiency of plating (marker rescue experiment)

	No prophage	λ*imm*434	Lysogen λ*imm*21	λ*imm*P22
λ*Bam*1	−−	++	++	++
λ*Jam*6	−−	++	++	++
λ*Nam*7	2×10^{-7}	1×10^{-5}	3×10^{-7}	4×10^{-7}
λ*Nam*53	1×10^{-6}	2×10^{-4}	1×10^{-6}	1×10^{-6}
λ*Oam*29	1×10^{-6}	5×10^{-3}	1×10^{-3}	2×10^{-6}
λ*Pam*3	3×10^{-8}	4×10^{-1}	1×10^{-2}	1×10^{-7}
λ*Pam*80	5×10^{-7}	2×10^{-1}	7×10^{-3}	7×10^{-7}
λ*Qam*73*Sam*7	$<10^{-8}$	3×10^{-2}	2×10^{-2}	8×10^{-3}
λ*Sam*7	−−	++	++	++
*Ram*5	−−	++	++	++

Lysogens were constructed in strain 594 which is strongly nonpermissive for *amber* mutants. Phage were plated either for efficiency (numbers) or as a serial-dilution spot test. Plates were incubated at 30° C since λ*imm*P22 carries a c_2ts allele. λ*imm*21 and λ*imm*434 were described by Herskowitz and Signer[21].

Table 2 Lefthand endpoint of nonhomology region



Frequency of recombinants

	*imm*P22	λ*imm*21	λ*imm*434
λ*bio*243	$<2\times10^{-7}$	$<2\times10^{-5}$	2×10^{-2}
λ*bio*10	2×10^{-3}	4×10^{-4}	—
λ*bio*252	1×10^{-2}	6×10^{-3}	

Crosses were done as standard λ crosses without ultraviolet-irradiation as described by Signer and Weil[33]. Recombinants were scored by their ability to grow on *E. coli* lysogenic both for P2 (which selects *bio*) and λ (which selects against *imm*λ). None of the parental phages grown in parallel produced progeny capable of growth on the test dilysogen at a frequency in excess of 2×10^{-5}. In the diagram, solid line indicates λ DNA replaced.

were scored. Table 1 shows that functional alleles of mutations in λ genes *Q*, *S*, *R*, *B* and *J* can be rescued, whereas the functional alleles of mutations in λ genes *N*, *O* and *P* cannot.

The appearance of nondefective *imm*λ recombinants in the cases of *Q*, *S*, *R*, *B* and *J* mutants indicates that the λ*imm*P22 prophage contains either (1) the wild type allele from its λ parent, or (2) an analogous and functionally compatible allele from the P22 parent which is separable from *imm*P22.

Failure to find functional *imm*λ recombinants in the cases of *N*, *O* and *P* mutants indicates that the hybrid phage does not carry these genes from the λ parent, and that the analogous P22 genes in the hybrid phage cannot be separated from the P22 immunity region of the hybrid phage.

These results suggest that in λ*imm*P22 the immunity region of λ has been replaced by an analogous region from phage P22 (Fig. 1). The region of nonhomology (or noncompatible function) between λ*imm*P22 and λ in the hybrid apparently includes genes *N* to *P* on the λ genetic map. The analogous region in P22 (*imm*C and its flanking genes) has been found to contain genes with functions analogous to those of λ (gene control and DNA replication in addition to immunity specificity)[1,22,23].

We have performed additional crosses to determine in detail the lefthand endpoint of the nonhomology between phages λ*imm*P22 and λ. λ*bio*10*imm*P22 but not λ*bio*243-*imm*P22 recombinants can be formed, indicating that the region of nonhomology extends to between the endpoints of these deletions. Table 2 shows that the lefthand endpoint of nonhomology of phage λ*imm*21 falls within same region. Since the *imm*21 substitution replaces the λ *N* gene, these crosses provide independent evidence that the *imm*P22 substitution also replaces λ gene *N*.

Functional comparisons of λ/P22 hybrids

(a) λ*imm*P22 has the same immunity specificity as λ*imm*21. λ*imm*P22 hybrids establish and maintain lysogeny normally; less than 1% of the cells in a lysogenic culture are cured. These lysogens are immune to superinfecting phage of the same genotype. We tested various lambdoid phages for the ability to grow in λ*imm*P22 lysogens. We also tested the ability of λ*imm*P22 to grow in lysogens of these lambdoid phages. As Table 3 shows, λ*imm*P22 grows normally in all lysogens except those carrying prophages with the immunity of phage 21 or P22. Furthermore, λ*imm*P22 lysogens are immune to those phages carrying the immunity region of phage 21 or P22. This result implies that the *imm*C repressor and operators of phage P22 are functionally equivalent to the repressor and operators of phage 21 and different from those of all other lambdoid phages tested. It should be emphasised that the *imm*C region of P22 is sufficient to control the hybrid phage λ*imm*P22, even though two immunity regions (*imm*I and *imm*C) control wild type P22. This result means that *imm*C is probably the primary regulator of repression and immunity in P22.

(b) We have obtained λ/P22 hybrids with substitutions of regions other than immunity. To construct hybrids different from λ*imm*P22, the *F'lac*$^+$*pro*$^+$(P22) strain was infected with various defective λ mutants. For example, this strain was infected with λ carrying *amber* mutations in both genes *Q* and *S*. *amber*$^+$ recombinants with the host range of λ were selected by plating the progeny phage on *su*$^-$ nonlysogenic *E. coli;* recombinants were obtained at frequencies between 10^{-3} and 10^{-4}. These recombinants have less DNA than the *Q*$^-$*S*$^-$ parent, as judged from their ability to grow in the presence of chelating agents[25]. Most of these phages were able to grow in λ*imm*P22 or λ*imm*21 but not in λ lysogens, indicating that they had retained the immunity specificity of the λ parent. If these hybrid phages were formed by the minimal recombination event (a double crossover), they should carry the P22 region analogous to the *Q*–*S* region of λ. Since both phages in this region confer the specificity of late gene expression[1,25], these hybrids should contain the head and tail genes of λ and the late gene control region from P22. Preliminary studies (P. Toothman and

Table 3 Immunity of λ*immP22*

	F'*lac* (P22)	F'*lac*	No prophage	λ*immP22*	λ*imm*21 (b_5)	λ*imm*434	λ+
λ+	+	+	+	+	+	+	0
λ*immP22* (3)	0	+	+	0	0	+	+
λ*imm*21*c*I	0	+	+	0	0	+	+
λ*imm*21cIIh80	0	+	+	0	0	+	+
λ*imm*21*cIbio*252	0	+	+	0	0	+	+
λ*imm*21 (b_5)p4	0	+	+	0	0	+	+
λ*imm*434	+	+	+	+	+	0	+
Φ80	+	+	+	+	+	+	+
82	+	+	+	+	+	+	+
381	+	+	+	+	+	+	+
424	+	+			does not adsorb		

These experiments represent serial-dilution spot tests. + indicate an efficiency of plating of near 1 and 0 represents no plaques seen, to a sensitivity of less than 1 in 10^5. The F'*lac* strains are the same as those used to isolate λ*immP22*; the other lysogens are in strain 594. The λ*imm*21 phages are derived from 21*hyc*I (ref. 20) or from λ *b5*, where indicated

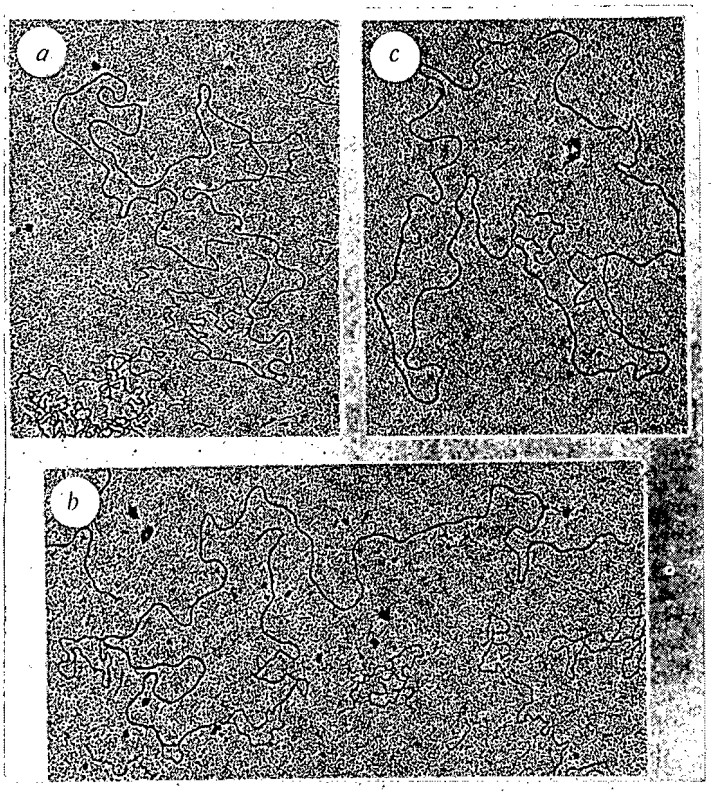


Fig. 2 Electron micrographs of DNA heteroduplexes prepared by the formamide method[34] from purified phage DNA. The b_2 deletion serves as a position and informal calibration marker. *a*, $\lambda b_2/\lambda imm$P22 heteroduplex molecule; *b*, $\lambda/\lambda imm21 b_2$ heteroduplex molecule; *c*, $\lambda imm21 b_2/\lambda imm$P22 heteroduplex molecule.

S. Hilliker, unpublished work) indicate that the λ late gene positive regulator (*Q* gene product) can turn on late genes from such prophages. The *Q* genes of λ and P22 may, therefore, have the same specificity.

Physical mapping of λ*imm*P22

The genetic tests presented above indicate that the λ*imm*P22 chromosome is essentially a λ chromosome in which the region from the left of gene *N* to some point beyond gene *P* of λ has been substituted by DNA encoding the analogous functions of phage P22. In addition, λ*imm*P22 and phage 21 have the same immunity specificity, suggesting that 21 and P22 share sequence homology in their immunity regions. To test these views directly, we constructed DNA heteroduplexes between all combinations of wild type λ, λ*imm*21 and λ*imm*P22. Each pair of DNAs differed also by a deletion (*b*2, in the central region of λ DNA) in order to orient the heteroduplex molecules and to provide an internal calibration for measurements. As Figs 2 and 3 show, the heteroduplexes confirm the genetic results: clearly λ*imm*P22 has a DNA substitution in the right arm of λ; λ DNA sequences are replaced by nonhomologous sequences from P22.

The regions of nonhomology between the three pairs of phages (λ/λ*imm*21, λ/λ*imm*P22 and λ*imm*21/λ*imm*P22) all begin at about the same point at the left (14.1—14.6 units from the *b*2 deletion). The *imm*21 and *imm*P22 substitutions therefore have approximately the same lefthand endpoint of nonhomology with respect to λ DNA. Since the *imm*21 substitution replaces the *N* gene of λ, the *imm*P22 substitution must likewise remove the *N* gene of λ. These conclusions confirm the results of recombinational studies shown above (Table 2).

The region of nonhomology between λ and λ*imm*21 is known to end to the left of λ genes *O* and *P* (ref. 26). The righthand endpoint of the major region of nonhomology between λ*imm*P22 and either λ or λ*imm*21 extends well beyond the point of nonhomology between λ and λ*imm*21 (Figs 2 and 3). Comparison with the physical map of Davidson and Szybalski[26] indicates that λ*imm*P22 must have DNA nonhomologous with λ extending to the right of λ gene *P*. Therefore, as suggested by earlier genetic results, λ*imm*P22 has a substitution covering the λ DNA replication genes and origin of replication. We show below that λ*imm*P22 behaves as if it has a *P* gene different from that of λ.

Three small regions of nonhomology between λ*imm*P22 and either λ or λ*imm*21 are found at the far right of the DNA heteroduplexes. The last of these is about 10.0 λ units from the right end of the DNA. If λ*imm*P22 were formed by only two crossovers between λ and P22 DNA, then the crossover on the right must have occurred somewhere to the right of this point. If this be the case, the existence of homology to the left of this point (between the small bubbles) suggests that there is natural homology between P22 and λ DNA in this region. Alternatively, λ*imm*P22 may have been formed by multiple crossovers between λ and P22 DNAs, such that only the three small nonhomologous regions are from P22. We favour the view that λ*imm*P22 resulted from two crossovers, since DNA–DNA hybridisation measurements[4] indicate that λ and P22 DNAs share 18% homology in the right arm.

Whereas the heteroduplex between λ and λ*imm*P22 shows a large region of nonhomology beginning at λ gene *N*, the heteroduplex between λ*imm*P22 and λ*imm*21 shows only a short region of nonhomology. To the right of this region is an equally short region of homology followed by another large region of nonhomology. The existence of a homologous region implies that the *c*I region of λ*imm*21 and the *c*2 region of λ*imm*P22 are not only functionally similar, but are also coded by homologous sequences in the DNA. In support of this placement of the homology to the repressor genes of the two phages, recombinants have been obtained with crossovers between the operators (S. Hilliker, personal communication). The repressor gene sequence homology suggests that the repressors of phages P22 and 21 have a common ancestry.

Control and replication genes

Physical and genetic mapping indicates that the *imm*P22 substitution replaces λ genes *N*, *O* and *P* with analogous genes (24, 18 and 12) of P22 (Fig. 1). This phage can, therefore, be used to study the behaviour of these P22 genes in an *E. coli* host. We have found that λ*imm*P22 hybrids grow normally in certain *E. coli* mutants (*gro*P$^-$, *gro*N$^-$, and *nus*$^-$) which fail to support λ growth (Table 4).

*Gro*P$^-$ mutants of *E. coli* have alterations of a host replication function (*dnaB*) which apparently must interact with the λ *P* gene product for successful λ replication[27]. Since λ*imm*P22 grows in these mutants, the P22 gene product analogous to *P* gene product either interacts with *dnaB* in a different manner or perhaps not at all. It should be noted that $groP_\lambda$ and $groP_{\mathrm{B}}$ mutations, known to lie in the

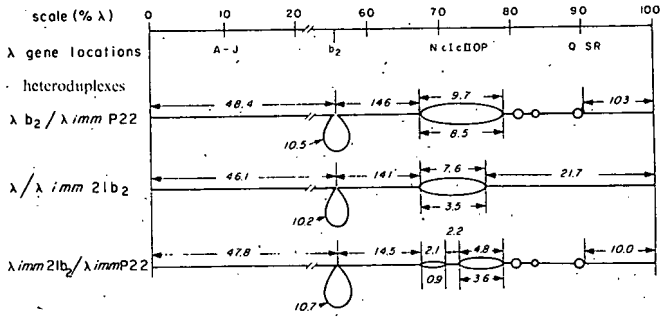


Fig. 3 Summary of measurements of DNA heteroduplexes. All the numbers are averages of at least eight molecules and are normalised to the total length of λ DNA. The locations of the λ genes are taken from Davidson and Szybalski[26].

Table 4 Efficiency of plating on bacterial mutants unable to support growth of λ

Host	λ+	λ*imm*P22	λ*imm*21
gro$^+$	(1)	(1)	(1)
groP$_{A15}$ (*dnaB*)	2×10^{-6}	1.1	5×10^{-6}
groP$_{B560}$ (*dnaB*)	2×10^{-6}	0.7	3×10^{-6}
groP$_{AB756}$	2×10^{-6}	0.07	3×10^{-5}
groN$_{785}$	2×10^{-6}	1.1	0.9
nus$^+$ (42° C)	(1)	(1)	(1)
nus (42° C)	2×10^{-6}	1.0	1.0
nus (30° C)	1.0	0.7	0.7

The *groP* and *groN* mutants are described in refs 27 and 29. *nus* strains are described by Friedman [30]. Figures in parentheses indicate normalisation to this value. All strains are nearly isogenic with either *gro*$^+$ or *nus*$^+$. Two independent λ*imm*P22 hybrids were tested.

dnaB gene[28], do not affect growth of λ*imm*P22, whereas the *groP*$_{AB}$ mutation, which is known to lie outside *dnaB*. (I.H., unpublished result), might have some effect on growth of the hybrid phages.

E. coli mutations *gro*N$^-$ or *nus*$^-$[29,30] block early λ transcription, probably by affecting the function of the early gene positive regulator, N protein. λ*imm*21 is able to grow in these bacteria (Table 4); λ*imm*P22, likewise, grows normally in *gro*N$^-$ and *nus*$^-$ mutants under conditions completely restrictive for λ growth, indicating that the early genes are regulated differently from λ.

From the behaviour in these *E. coli* mutants, it is clear that the early gene control and DNA replication genes of P22 have specificities distinctly different from λs when assayed in the same genetic background.

Implications for virus evolution

The formation of viable hybrids between coliphage λ and the *Salmonella* phage P22 shows that the similarity between these two phages in the arrangement of functions on their genetic maps is the result of a substantial degree of common evolutionary ancestry. We imagine that the hybrid phages were formed by phage-specified or bacterial recombination rather than "illegitimate recombination"[31] because of the relatively high frequency with which they are formed. Not only are viable hybrids formed, but the immunity specificity of P22 and phage 21 are identical, both in function and, to the resolution afforded by heteroduplex mapping, in nucleotide sequence. Similarly, the *Q* gene of λ and the analogous gene (gene 23) of P22 seem to be functionally identical.

A second striking fact about these hybrids is the extent to which nonhomologous genes controlling DNA replication, regulation and immunity of phage P22 remain fully functional when inserted into the chromosome of phage λ. Also notable is the retention of regions of homology flanking nonhomologous segments of DNA which contain nonidentical genes such as those controlling DNA replication. The conclusion which emerges is that evolution of the lambdoid phages (which must now include P22) appears to have proceeded within regions of function which have been selected for the ability to form viable combinations with functional segments from other members of the lambdoid phage group. Since P22 and λ have different bacterial hosts, selection must have maintained this functional and recombinational compatibility for at least the time in evolution since *E. coli* and *S. typhimurium* diverged. It should be noted that *Salmonella* and *E. coli* can exchange genetic material and have similar genetic organisation[35]. Perhaps the enteric bacteria have themselves evolved by a mechanism similar to that suggested for bacteriophages.

Our view of bacteriophage evolution depends on the clustering of related functions on the genetic map[31–34]. The idea that such clustering might be selected through hybrid formation has been advanced as well. But, the idea that the lambdoid phage "races were derived from still other races by re-assortment of unlike segments" the concomitant idea that "presumably both the diversity [of the functional segments] and the capacity to form hybrid recombinants have served a joint evolutionary purpose" was first clearly formulated by Hershey[32]. This idea must now be taken to extend across the species lines of the enteric bacteria.

Heteroduplex studies of the DNA of the lambdoid phages[36,37] and of the lytic T series of coliphages[38,39] support this view of bacteriophage evolution. One can see in the heteroduplexes the whole range of evolutionary divergence: at some points, entire regions of function are nonhomologous and at other points the region of nonhomology may involve only a single gene or even a part of a gene[40]. Although the principle of exchange of functional regions might apply to all bacteriophages, not all phages seem to be in the same group. For example, the temperate coliphages P2 and 186 form viable hybrids with each other (unpublished results of B Egan, H. Younghusband and R. B. Inman) but not, thus far, with the lambdoid phages; similarly, the lytic coliphages T3 and T7 appear to belong to a group separate from that containing phages T2, T4 and T6.

In conclusion, we suggest that evolution proceeds by diversification within segments encoding particular functions: each segment retains functional compatibility and recombinational homology so that reassortment among them is easy and frequent. We imagine that this idea might apply quite generally to bacteriophages, and, indeed, to all viruses, including those of mammals. One might expect therefore that functional hybrids among viruses of quite divergent host species might frequently be encountered.

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LETTERS TO NATURE

Distance to Cygnus X-1

THERE has been much discussion concerning the X-ray object Cygnus X-1 and its optical counterpart, HDE226868, a highly reddened star of type O9.7 Iab (ref. 1). The O star is a single-lined spectroscopic binary, and various assignments of the minimum mass of the secondary, taken to be the X-ray object, suggest that the latter is a black hole. These conclusions are dependent on the mass of the O star being $\sim$ 20–30 $M_{\odot}$, as would be the case if the star were 'normal' for its luminosity class. But the possibility of heating of the primary by the nearby X-ray object, and the spectroscopic indications of mass loss[2], indicate that such an assumption is unsafe. In order to determine the mass of HDE226868, a more complete spectroscopic analysis, in which the effective temperature and surface gravity are deduced, and a determination of luminosity (from the distance to the object) are necessary. The distance to the object is crucial to the problem, because once the effective temperature and gravity of the O star are known, the mass will vary as the square of the distance. An error in the distance by a factor of about two means that the model for the X-ray source need not include a black hole.

Accurate estimates of the distance to HDE226868 have recently been claimed by Margon *et al.*[3] and Bregman *et al.*[4] who give 2.5 kpc. Such a large distance favours the black hole model. Both works used the reddening produced by interstellar dust as a distance indicator. A consistent distance–reddening relationship has been assumed for this region of the sky. The evidence for this needs to be examined closely.

Interstellar reddening is particularly irregular in the Cygnus region. The absorbing clouds of dust collectively form the Cygnus Rift, in which there are many highly reddened OB stars. Bregman *et al.* showed that HDE226868 is highly reddened compared to the objects—mostly F stars—in their small field of view. Apart from the fact that HDE226868 is located extremely near, or actually within, the Great Rift, it is clear that it has photometric properties very similar to reddened O stars in this same region surveyed by Johnson and Morgan[5]. This, of course, does not necessarily imply that the object is a normal O star close to the main sequence. Plotting the colours of HDE226868 measured by Bregman *et al.* ($B-V=+0.81$, $U-B=-0.30$) on a reproduction of Johnson and Morgan's two-colour diagram (Fig. 1), shows that it is not anomalous: there are many O stars in this area which are more highly reddened.

The relationship between total extinction, A_v (commonly taken to be $3E(B-V)$), and distance for fields in the vicinity of the Milky Way has been investigated by Neckel[6]. Neckel was unable to deduce any relationship between A_v and distance within a region centred on $l^{II}=73°$, $b^{II}=0°$, which is extremely close to HDE226868. That survey is, however, considered by Margon *et al.* to be inapplicable because Neckel's fields were wide enough to include regions where large changes of absorption could occur over small angular scales. Margon *et al.* attempted to overcome this difficulty by selecting stars within 50′ of

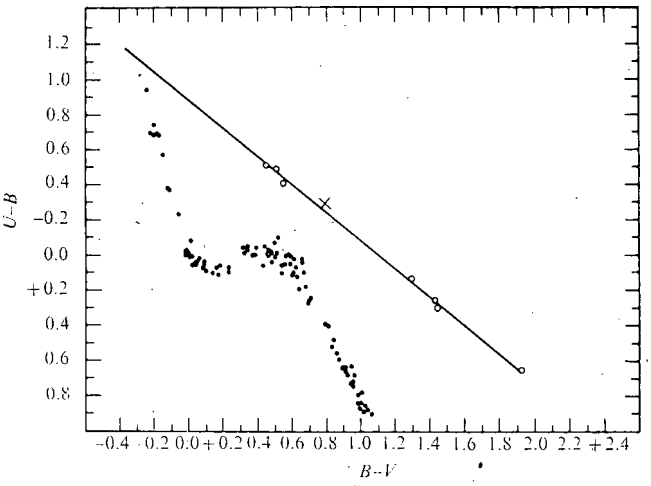

Fig. 1 Two-colour plot for reddened O stars in Cygnus from Johnson and Morgan[5]. ●, unreddened main sequence stars (from types B1 to K3); ○, O stars in the Cygnus Rift Region; × HDE226868, from the colours of Bregman *et al.*[4].

HDE226868. This procedure was clearly not successful, because even at distances below 1 kpc there is an extremely large scatter of points in their plot. The extrapolation of such data to greater distances is thus hardly justified. The same applies to the plot of $E(B-V)$ against distance for the survey of stars[4] within 30′ of HDE226868. In this case, an independent distance check is provided by the Cepheid V547 Cyg, for which $d=6.6$ kpc, about two and a half times greater than would have been estimated from the assumed $E(B-V)$ against d relationship.

We therefore conclude that this region is extremely patchy over angular scales as small as 1°. This is confirmed by Lynds'[7] survey of dark nebulae which shows not only general patchiness but also the presence of extremely small dark nebulae. The space density of these tiny nebulae may, moreover, be underestimated because of the lack of sufficient background illumination. If these objects are representative of the familiar 'Bok globules', then many may have linear dimensions of only 0.05 pc (ref. 8), and would therefore subtend only 10″ at a distance of 1 kpc. As the reddening produced by the globules is as high as $A_v \sim 5$ magnitudes, large extinction changes can occur over areas of sky which are very much smaller than those covered by either Margon *et al.* or Bregman *et al.*

Because of the extremely complex nature of the Cygnus region, and the fact that the only independent distance check is so widely in error, the quotation of a distance to ± 16% by Margon *et al.* cannot be taken seriously. At present we consider that it is over-optimistic to expect reddening measurements to be able to provide the distance of HDE226868 to the required accuracy. Until there is a far more accurate determination of the distance of this object it seems premature to suggest that the X-ray source is a black hole.

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High energy radiation from white holes

COMPARED to black holes, their time-reversed versions, white holes have attracted little attention from theoreticians. Here we point out possible ways in which white holes may be useful to high energy astrophysics.

Basically a white hole is an object exploding from a highly dense or a singular state. It may be a case of delayed big bang[1] in a Friedman universe, or it may be an instance of a collapsing object reversing implosion to explosion. For the latter possibility unconventional equations of state[2] or negative energy fields[3] are required if the theoretical framework is that of general relativity[4]. We will take the white hole to be an object exploding from a singularity and obeying Einstein's equations of gravitation subsequent to the singular event. Before turning to the astrophysical implications we calculate the spectral features to be expected from a white hole.

For simplicity we make the following assumptions: (1) The white hole emerges from the singularity as a spherical object of uniform density and zero pressure in the comoving frame of the outward moving particles; (2) the light emitted by the white hole is monochromatic and is being emitted radially outwards from the surface at a uniform rate in the comoving frame of reference; (3) the space-time exterior to the object is empty.

The comoving frame of reference in the interior will be denoted by coordinates (r, θ, Φ, t) in terms of which the line element within the object is given by

$$ds^2 = c^2\,dt^2 - S^2(t)\,[dr^2/(1-\alpha r^2) + r^2(d\theta^2 + \sin^2\theta d\Phi^2)] \quad (1)$$

where c = speed of light, $S(t)$ is the expansion factor and α a parameter related to the mass M and the comoving radius r_b of the object by

$$2GM = \alpha r_b^3 c^2 \quad (2)$$

The similarity of equation (1) to the Robertson–Walker line element of cosmology is well known. Also, if we change t to $-t$, equation (1) represents a freely collapsing ball of dust.

For convenience we will measure t from the instant of explosion so that $S(0) = 0$. For $t > 0$, $S(t)$ satisfies the equation

$$S\dot{S}^2 = \alpha c^2 (1-S) \quad (3)$$

so that it attains its maximum value $S = 1$ at

$$t = t_0 = \pi/(2c\sqrt{\alpha}) \quad (4)$$

We will investigate light emission from the white hole in the interval $0 < t < t_0$.

The space exterior to the white hole is described by the Schwarzschild line element

$$ds^2 = [c^2 - (2GM/R)]\,dT^2 - dR^2/[1-(2GM/c^2R)] - R^2(d\theta^2 + \sin^2\theta d\Phi^2) \quad (5)$$

A typical Schwarzschild observer has R = constant, θ = constant, Φ = constant. We wish to calculate the spectrum of radiation from the white hole as seen by a Schwarzschild observer with $R = R_1 \gg 2GM/c^2$. In accordance with our assumption (2) we will take the luminosity spectrum of the white hole as $L\,\delta(\nu - \nu_0)$, where L = constant.

To calculate the spectrum received at R_1, define

$$S = \sin^2\theta,\ 0 \leqslant \theta \leqslant \pi/2 \quad (6)$$

Then θ as a function of comoving time coordinate t is given by

$$t = (2t_0/\pi)(\theta - \sin\theta\cos\theta) \quad (7)$$

The white hole bursts out of the Schwarzschild radius at $t = t_c$, $\theta = \theta_c$, where

$$\sin\theta_c = (\alpha r_b^2)^{\frac{1}{2}} \quad (8)$$

Suppose two successive light signals are sent out from the surface at comoving instants t and $t + dt$ and are received by the observer at R_1 at instants T and $T + dT$ measured in the Schwarzschild coordinate. Then a straightforward calculation shows that

$$dT/dt = \sin\theta/\sin(\theta + \theta_c) \quad (9)$$

So an electromagnetic wave of frequency ν_0 emitted from the surface appears to the receiver to have the frequency

$$\nu = \nu_0 \, [\sin(\theta+\theta_c)/\sin\theta] \qquad (10)$$

A result of this type but in different forms had been obtained earlier by others[5,6] but the above form is suitable for working out the spectrum of the radiation as seen by the Schwarzschild observer. Under our assumption (2), $L/h\nu_0$ photons of frequency ν_0 are being emitted per unit t-time from the surface. The number emitted in the interval $[t, t+dt]$ is therefore $Ldt/h\nu_0$. The same number must be received in the interval $[T, T+dT]$, but with frequencies in the range $(\nu, \nu+d\nu)$ where $d\nu$ is related to dt through equations (7) and (10).
A simple calculation gives

$$dt = (4t_0\nu_0^3 \sin^3\theta_c \, d\nu)/(\pi \, (\nu^2+\nu_0^2-2\nu\nu_0 \cos\theta)^2) \qquad (11)$$

Writing $E = h\nu$, $E_0 = h\nu_0$, the number of photons in the range $[E, E+dE]$ received from the white hole per unit area at $R = R_1$ is given by

$$N(E)dE = (Lt_0/\pi^2R_1^2)E_0^2\sin^3\theta_c dE/(E^2+E_0^2-2EE_0\cos\theta_c)^2 \qquad (12)$$

For $E \gg E_0$

$$N(E) \, dE \cong Lt_0 \, E_0^2 \, (\sin^3\theta_c/\pi^2 \, R_1^2) \, dE/E^4 \qquad (13)$$

The energy spectrum $I(E)$ is given by

$$I(E) = EN(E) \propto E^{-3} \qquad (14)$$

This is the spectrum at the high energy end under the simplifying assumptions made here. More general (and perhaps more realistic) assumptions can lead to different types of spectra which we have also worked out. We now wish to indicate possible fields in high energy astrophysics where white holes may find applications.
(i) The hard electromagnetic radiation from white holes situated at the centres of, say Seyfert galaxies, can be a source of background X and gamma radiation. The energy spectrum (14) seems, at first sight to be too steep compared to the observed[7] spectrum $\propto E^{-1.2}$. But absorption effects in the gas present in the nuclei surrounding the white hole tend to flatten the spectrum given by equation (14). Detailed calculation with available data[8,9] shows that these absorption effects can in fact flatten the E^{-3} spectrum to $\sim E^{-1}$ form in the range 0.2 keV to 1 keV. At lower energies, the ultraviolet radiation seems to be of the right order of magnitude to account for the infrared emission of $\sim 10^{45}$ erg s^{-1} through the dust grain heating mechanism[10].
(ii) The transient nature of X-ray and gamma-ray bursts[11] suggests a white hole origin. The shape of the spectrum at the emitting end is likely to be more complicated than the very simple form assumed in the above example. In general, however, the spectrum should soften with time.
(iii) Although we have worked out the spectrum of photons, it is not difficult to see that similar conclusions will apply to particles of non-zero restmass provided they have very high energy. It is possible therefore to think of white holes in the Galaxy on the scale of supernovae, yielding high energy cosmic rays right up to the highest energy observed.

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Spectrum of the cosmic background radiation between 3 mm and 800 μm

WE report the successful flight of an experiment designed to measure the spectrum of the cosmic background radiation from ~3 mm to wavelengths shorter than the Planck peak for a temperature of 2.7 K. The experiment was launched, to an altitude of 40 km, on March 13, 1974, from Palestine, Texas. Because of an on-site computer fault, the complete data were not available to us until May 7, 1974. The instrument has been described previously[1,2] and will be discussed in detail in a future paper; we present here the results of preliminary analysis of the flight data.

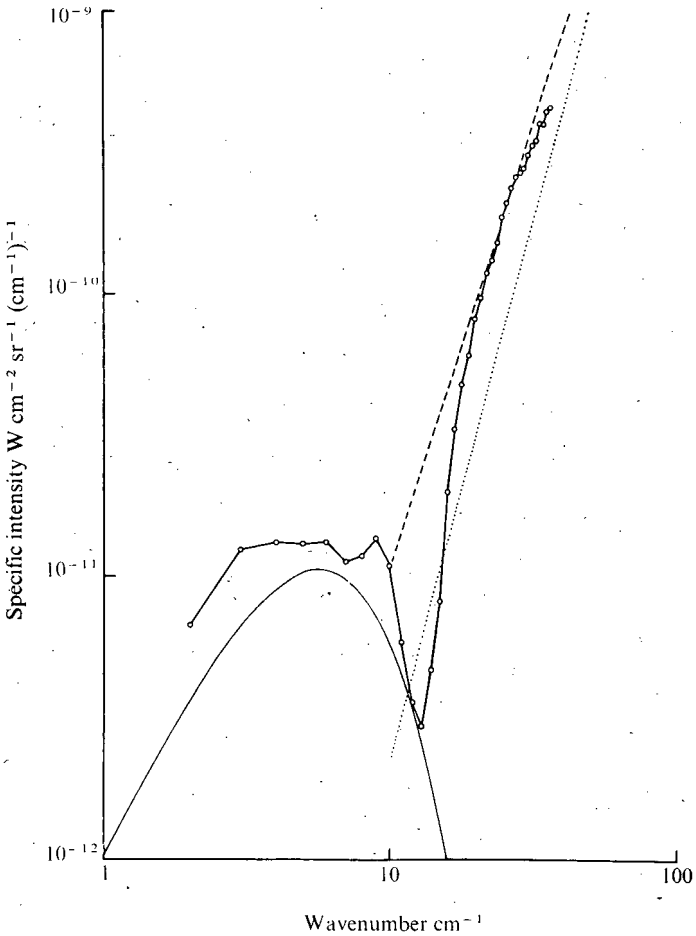

Fig. 1 Cosmic background spectrum at 1 cm^{-1} resoluton: ——o——, Observed spectrum; — — — —, predicted window emission; . . . . . , window emission using the results of Chantry *et al.*[4]. Smooth solid line denotes 2.7 K minus 1.4 K curve.

The instrument consists of a polarising Michelson interferometer[3] cooled, by a liquid helium bath, to a temperature of 1.4 K. The processed output of the interferometer is the difference between the spectrum of radiation entering the cryostat and the spectrum of the radiation within the cryostat cavity. The interferometer received radiation at a zenith distance of 50° within a cone of semi-angle 3°; the zenith distance of the extremity of the balloon, as seen from the package, was 27°. The interferometer operated in vacuo, separated from the atmosphere by a 50 μm thick window of polyethelene; the measured spectrum should therefore consist of the cosmic background radiation plus emission from the atmosphere and the window. The designed (unapodised) spectral resolution was 0.25 cm^{-1} (Rayleigh criterion). The instrument was calibrated in the laboratory using simulated black body sources at room, liquid nitrogen, liquid hydrogen and liquid helium temperatures; for in flight calibration, we decided to use the emission from the window. The efficiency of the instrument, as a function of frequency, was deduced from the observed room-temperature laboratory spectrum.

One hour of useful time was achieved at float altitude (reached at 0145 CDT), giving 16 interferograms. Total integration time for each sampled point was 19 s. The spectra obtained from these interferograms were averaged at 1 cm^{-1} resolution; the result was divided by the instrumental efficiency function and the high frequency part fitted to the expected window emission between 20 and 30 cm^{-1} to establish a vertical scale. The fitted spectrum is shown in Fig. 1. Also shown is the spectrum of à 2.7 K black body (representing the supposed cosmic background) minus the spectrum of a 1.4 K blackbody (representing the interior of the interferometer cavity).

It is not clear why, below 20 cm^{-1}, the observed spectrum of emission from the window departs radically from that predicted. The prediction of emission, however, is difficult because of the problems of measuring absorption in thin samples. Estimates of absorption vary between experimenters; for example, we show in Fig. 1 the predicted window emission using the absorption figures of Chantry *et al.*[4]. We believe, however, that the measured values (E. I. Robson and B. Carli, to be published) used for fitting are correct to $(+20, -60)\%$. It is seen that, if the predicted emission were decreased appreciably, the measured low frequency spectrum would fall below that of 2.7 K minus 1.4 K blackbodies. We have tried the effect of assuming higher temperatures for the interferometer cavity, but to obtain any significant reduction in the height of the predicted background curve requires that the peak emission be displaced to such high frequencies as to exceed the measured values. We are therefore satisfied that the calibration is substantially correct.

Our measured spectrum is a factor less than ~2 higher than that of a 2.7 K background at frequencies below 10 cm^{-1}. A temperature $\sim 2^{1/3}$ higher than 2.7 K is ruled out, however, by the position of the peak. The excess may well be due to unresolved atmospheric emission[5]. In any case, this first direct spectral measurement shows very clearly the sharp decline in the spectral intensity above 6 cm^{-1}, and it would be very difficult to reconcile our observation with a background temperature higher than ~2.9 K.

We thank our colleagues at Queen Mary College who have contributed to this experiment and the staff of the National Center for Atmospheric Research Balloon Facility for their expert assistance. The project was supported by a grant from the Science Research Council. E.I.R. and J.S.H. acknowledge an SRC Associateship and Studentship respectively.

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A new solar–terrestrial relationship

RECENT suggestions of interrelationships between solar activity, the Earth's magnetic field and the weather[1–3] make more urgent the need to have a reliable method of predicting sunspot activity.

Most attempts to develop prediction techniques have been associated with empirical relationships derived from the preceding behaviour of many sunspot cycles, but none of these has been really successful over any extended period. Wood[4] has re-opened consideration of the possibility[5] that solar activity may be controlled by the tidal influence of the planets. In the course of a detailed study[6] of 25 yr of geomagnetic data it was found that the phase of the solar diurnal variation of the horizontal field on quiet days, Sq(H), exhibits a marked solar control: the variability of the phase is most marked in winter and at sunspot minimum epochs. The solar cycle behaviour has now been studied over an 89-yr period from 1884, the earliest year for which International Quiet Days have been designated, and a consistent tendency has been found for the magnetic data at sunspot minimum to anticipate the magnitude of the subsequent sunspot maximum.

Both the amplitude and phase of the Sq variation vary considerably from day to day, even on very quiet days. The variability in the amplitude has been well studied, but relatively little work has been published on the variability in the phase. Here, the phase variability is discussed. Figure 1 shows the diurnal variation of the occurrence of the time of daily minimum horizontal field for all the international quiet days in the period 1884–1972. Although the most common time of *H*-min is between 1030 and 1130 LT, there is a wide range of appropriate times. Days for which H-min occurred within 2½ h of 1100 LT

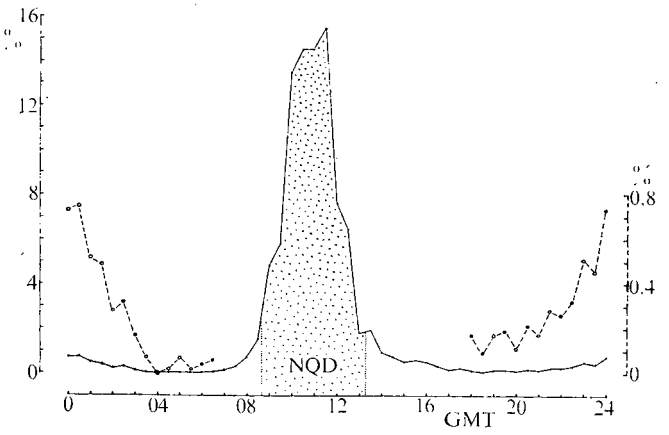

Fig. 1 Diurnal variation of the percentage number of international quiet days of the period 1884–72 for which the daily minimum horizontal magnetic field occurred within each half-hour of the day at Greenwich/Abinger/Hartland. Dashed curve is for night-time data plotted on a ×10 ordinate scale, indicated on the right-hand side.

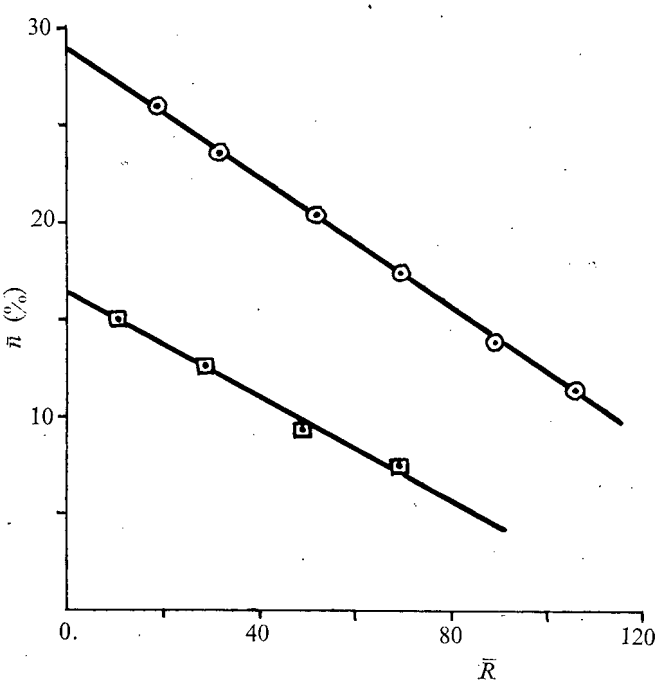

Fig. 2 Average percentage occurrence of AQDs ($\bar{n}$) as a function of average sunspot number (R) derived from annual mean 3-yr smoothed data averaged over increments of 20 in R. Data are the same as in Fig. 1, subdivided into two periods: moderate activity, 1885–1936 (squares); strong activity, 1937–71 (circles).

are styled 'normal quiet days' (see ref. 6). These are included within the shaded region marked NQD in Fig. 1. All days outside this region are defined as 'abnormal quiet days' (AQD).

The dashed curve in Fig. 1 represents a replot of the points for the night-time period on a $\times 10$ ordinate scale; it seems that within the AQD category there is a subsidiary maximum occurrence of near-midnight minima in H. At such times the conductivity of the E region of the ionosphere is so low that essentially no electric current flow can occur, so that the reduction in the horizontal magnetic field cannot be ascribed merely to positional changes of the atmospheric Sq current loops. Evidence is presented elsewhere[7] for a connection between the occurrence of the more moderate AQDs and changes in the structure of the terrestrial atmosphere and the orientation of the Sq current loops, but it seems likely that the more extreme types of AQD have an extraterrestrial (probably solar) cause.

frequency of AQDs with decrease in solar activity, such that the occurrence is primarily confined to times near solar minimum. Extrapolating the Fig. 2 data shows that the mean occurrence frequency of AQDs for 'zero sunspots' for the 1885–1936 cycles is 16.5%, and for the 1937–71 cycles it is 29%. These differences probably emphasise the relative crudity of the sunspot number as an index of solar activity. The earlier group is for a period of relatively low sunspot maxima and low minima; the later group has high sunspot maxima and relatively high sunspot minima, and it seems likely that the bodily displacement of the lines in Fig. 2 is conditioned by varying thresholds of solar activity which are not apparent in the values of sunspot numbers themselves. The 'AQD count' parameter is interesting since it is an index which maximises at sunspot minimum, and it is conceivable that it thereby provides a rather sensitive measurement of the 'depth' of solar minimum not evident in the conventional sunspot number.

Further insight into the solar cycle control of AQD occurrence is provided by an examination of the long-term behaviour over the eight separate solar cycles investigated. Figure 3 shows a 3-yr running mean of the average quarterly values of AQD count (solid curve) and sunspot number (dotted curve). The inverse correlation between the two curves corroborates the result of Fig. 2; a new feature is the striking variation in the amplitude of the maxima of the AQD count (at sunspot minima) such that the change from one cycle to the next is always in the same sense as the change in amplitude of the subsequent sunspot maxima. This effect is better illustrated in Fig. 4 which shows 3-yr running mean curves of the annual values of both parameters, plotted so that the time scale for sunspot number is 6 yr ahead of that for the AQD count. The effect is consistently observed over the ups and downs of the past eight solar cycle maxima, and is particularly evident over the period 1920–72 when after three consecutive increases in the size of the AQD count and the subsequent solar maxima (culminating in the record IGY maximum) the following maximum was significantly smaller for both parameters.

It is now possible to rationalise the two lines obtained in Fig. 2 when concurrent values of AQD count and sunspot number are plotted against each other. Figure 5 shows a plot of the amplitudes of the cycles in each parameter, read from Fig. 4, using both the ascending and descending parts of each cycle, where each AQD count amplitude is associated with the corresponding sunspot number amplitude one half-cycle ahead. The approximately linear relationship shows that each change in AQD count is approximately mirrored by a proportional change in sunspot number during the corresponding part of the subsequent cycle.

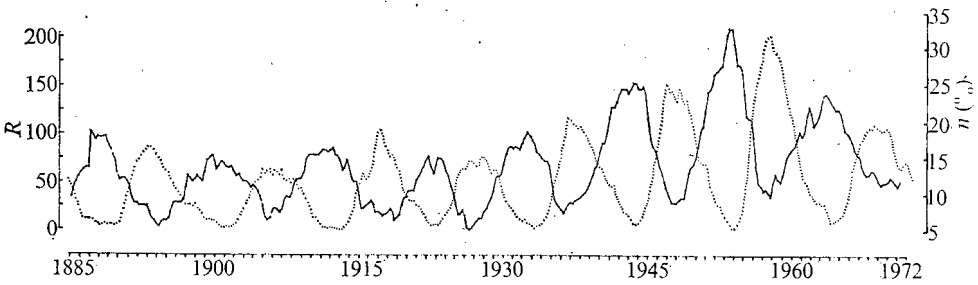

Fig. 3 Three-yearly running mean of the quarterly occurrence of AQDs (n, solid curve) and of sunspot number (R, dotted curve) for the data as in Fig. 1.

It was previously found[6] that the occurrence of AQDs is primarily a winter phenomenon, and examination of the detailed seasonal variation over the period 1884–1972 shows that the most extreme AQDs occur exclusively in winter. Confirmation of this inverse relationship to solar radiation is provided by the negative correlation between AQD occurrence and sunspot number, illustrated in Fig. 2. In this diagram the data have been smoothed over 3 yr and the mean AQD count determined for each successive interval of 20 in sunspot number.

For each group of data in Fig. 2 (1885–1936 and 1937–71) there is a remarkably linear increase in the occurrence

If this relationship proves valid it implies that the Sun 'breathes' with an 11-yr period, such that the size of a solar activity maximum is determined at the very beginning of a cycle, or perhaps the very end of the preceding cycle, from the 'depth' of the solar minimum. This quantity is essentially unmeasurable by the conventional sunspot number, and may be insensitive of detection by other indices of solar activity which decrease at this time. The AQD count index, based on a simple analysis of the variation of the Earth's magnetic field on quiet days in winter, seems to provide a highly sensitive index of the extent of solar minima. Detailed examination of the various cycles in

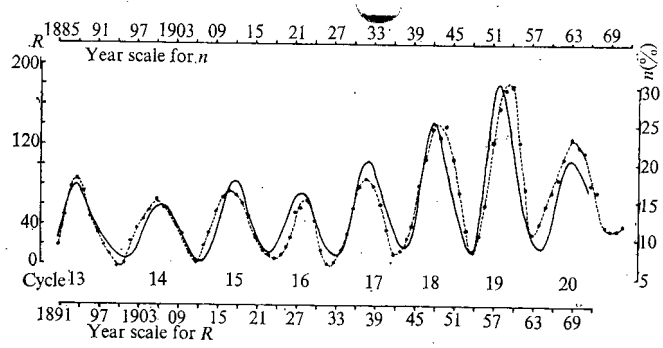

Fig. 4 Three-yearly running mean of the annual occurrence of AQDs (n, dashed curve, upper abscissa scale) and of sunspot number (R, solid curve, lower abscissa scale) for the data as in Fig. 1. Note the 6-yr displacement of the time scales.

Fig. 3 reinforces the conclusion that an apparently close relationship exists between the two parameters separated by an interval of 5–6 yr. For example, there is a marked tendency for some maxima in AQD count to be double-humped, with peak separations of about 2 yr. Double maxima are a feature[8] of the solar cycle (although not always evident in sunspot number) and have been interpreted as the result of the superposition of two processes having different physical properties. It is tempting to suggest that the extra large and single-humped sunspot maximum of 1957–58 arose from a coincidence of the two peaks, and it is noteworthy that the preceding AQD count maximum in 1953–54 also exhibits a single sharp peak.

There are obvious difficulties in accounting for a time constant as long as 5–6 yr from the solar side. But it is now evident that the solar cycle is a much more complicated periodicity than that indicated by the variation of any one index of activity. The cycles in solar wind intensity and velocity, coronal green line intensity and coronal shape, and sunspot number all differ in phase from one another, with a maximum difference of at least 3 yr (ref. 9). One model[10] of the Sun's magnetic field proposes that the magnetic polarity of solar polar regions changes sometime during the decline from sunspot maximum to sunspot minimum, and a solar activity centre is considered to have a 'history' related to solar happenings during the preceding years. The interplanetary field near the Earth is largely controlled by the solar polar field and, in keeping with the above result, the phase of the annual variation of the interplanetary field changes about 2.7 yr after sunspot maximum[11].

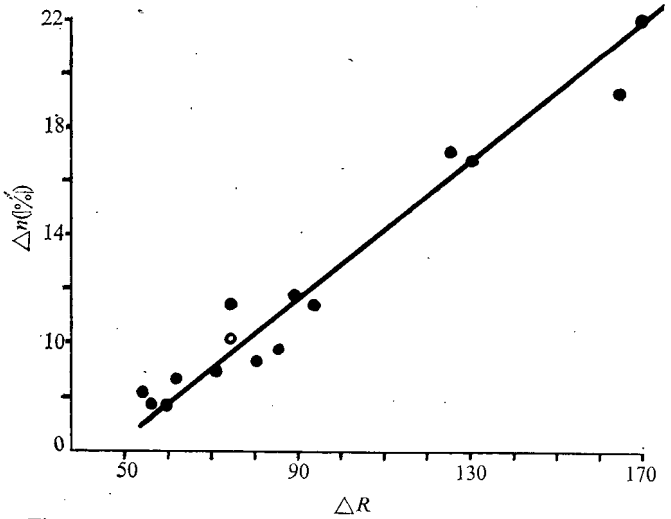

Fig. 5 Amplitudes of the cycles in AQD occurrence ($\triangle n$) and sunspot number ($\triangle R$) one half-cycle ahead, read from Fig. 4. (Open circle is for the ascending part of cycle 13 and involves an estimate of the value of $\triangle n$, since no magnetic data are available for the time of the AQD minimum.)

From the terrestrial magnetic side it is difficult to speculate on possible mechanisms while the essential cause of the AQD phenomenon remains obscure. Comparison of Sq data for observatories spaced 1,000 km apart has shown[6] that 80% of the AQDs are common at the two sites in winter and at sunspot minimum; the agreement falls to 35% in summer and sunspot maximum. This suggests that when the most variability is encountered it influences larger areas of the Earth. Thus, although the AQD index, as presented, is derived from data for one station only, it reflects a regional or planetary phenomenon. The unusual minima in the horizontal magnetic field at a station tend to occur around 0000 UT and are generally additional to the normal minima around 1100 LT which are associated with the ionospheric Sq current system[12]. So it seems very likely that the cause of the AQD incidence is extraterrestrial, and is probably solar in origin. A likely line of communication between the Sun and Earth seems to exist in the interplanetary field. The varying 'sector structure' of this field, which is related to the solar magnetic field, can be seen clearly in the behaviour of the geomagnetic field at high latitudes[13]. It has also been proposed[14] that during quiet days the magnitude of the total interplanetary magnetic field is partly responsible for the day-to-day variability of Sq (range rather than phase) at low latitudes. Furthermore, the sector structure of the interplanetary field shows a solar cycle variation[15], and exhibits a particularly stable structure at sunspot minimum[16].

There remains the possibility that the relationship reported here is fortuitous, albeit being obeyed for a tantalisingly long period. The ultimate test must await much more extensive data, or the realisation of a sound scientific basis for such a connection along the lines suggested above. Meanwhile, the index could probably be refined considerably by basing it on magnetic data from several geographically separated stations, and by defining the incidence of an AQD more precisely. It seems (Fig. 4) that there is a long term secular trend in AQD occurrence, evidenced by the almost continual increase in the size of the minimum count of each cycle over the period covered.

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Rainfall, drought and the solar cycle

THE present drought devastating the Sahel, India and Ethiopia has been ascribed to long term climatic changes. Winstanley[1] and Lamb[2] suggest that the general circulation of the atmosphere is slowly changing, resulting in an equatorward shift of the principal climatic belts. If these shifts are part of a 200-yr cycle, as Lamb suggests, severe droughts should become more

common during the next 60 yr. But at Addis Ababa the 11-yr solar cycle seems to have a greater influence on rainfall variations and so the timing of droughts, than does the long term cycle.

In Fig. 1 we compare the variation of annual rainfall recorded at Addis Ababa during the past 72 yr (refs 3–5 and unpublished data from Addis Ababa Geographical Observatory) with the Zurich sunspot number. Both sets of data have been smoothed by plotting 3-yr running means. The rainfall clearly follows a cyclic pattern in which rainfall peaks and troughs precede the sunspot peaks and troughs by a few years. This agreement is not caused by the smoothing technique used: comparing each year's unsmoothed rainfall and sunspot values the correlation coefficient, r, is 0.21 (significant near the 5% level). If each year's sunspot value is compared with the previous year's rainfall, $r=0.29$ (significant near the 1% level) and if compared with the rainfall 2 yr before, $r=0.33$ (significant at better than the 1% level). Thus, there is a strong statistical correlation between Addis Ababa rainfall and sunspot number, the rainfall peaks leading the sunspot peaks by an average of 1.3 yr; the actual values for the 7 cycles being: 1, 2, 3, 2, 1, 1, 0 yr. It is noteworthy that, in a reported correlation between atmospheric ozone content and sunspot values[6], the ozone troughs preceded the sunspot peaks by 1.5 to 2 yr.

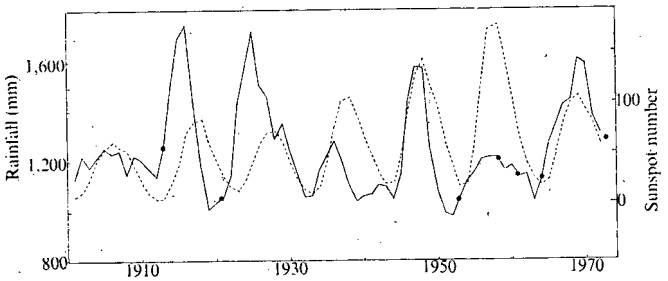

Fig. 1 Three-year running means of Addis Ababa rainfall (———) and three-year running means of Zurich sunspot numbers (— — —). ●, Known Ethiopian drought years. In cases where discrepancies occur between rainfall data from refs 3 and 5 preference has been given to ref. 3.

The phase relationship of the two curves of Fig. 1 is well maintained, but the amplitude relationship is more complicated. There is a tendency for the magnitude of the rainfall peak to be inversely related to the magnitude of the sunspot peak (Fig. 2*a*). A similar relationship appears for the trough values (Fig. 2*b*). But since two of the data points on each graph disagree with this trend there is not sufficient evidence to state this relationship definitely.

If this rainfall/sunspot cycle relationship continues then, using King-Hele's[7] prediction of 1978 as the peak year of the next solar cycle, we would expect Addis Ababa rainfall to increase until about 1976–77 and then decline to a minimum about 5 yr later. Further, if the relationship shown by Fig. 2*a* holds, then the predicted value of 110 for the next sunspot number peak[7] gives a value of about 1,600 mm for the next rainfall peak.

It is important to find whether such a relationship applies for Ethiopia as a whole, and for other drought afflicted areas of Africa. Instrumental rainfall records have been obtained only recently in most areas of Ethiopia, but a 70-yr record exists for Asmara. A preliminary study of this shows little correlation with solar cycle before 1930, but a negative correlation after that date.

To support the scanty instrumental data on rainfall, an indication of past rainfall troughs can be obtained from historical records of drought, and drought-induced famines. To be recorded by travellers or historians a drought would have to have been severe enough, and lengthy enough, to affect seriously the life of the country, and not have been merely an isolated year of poor rainfall. Figure 3 is a histogram of presently known Ethiopian drought years, since 1540 AD,

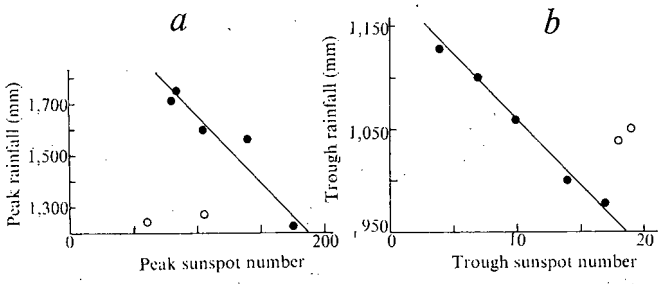

Fig. 2 *a*, Rainfall cycle peak values against corresponding sunspot cycle peak values. The line is a least squares fit to the solid points only. *b*, As for *a* but for corresponding trough values.

as a function of years from sunspot minimum. Droughts have occurred in each of the years of the sunspot cycle, but 14 of the 27 drought years occurred in the 3 yr centred on sunspot minimum. Six more occurred in the years following minimum, possibly representing droughts caused by an accumulation of bad years (the effects of a long deficiency of rain persist during the first years of improved rainfall). Thus, there is some evidence that the rainfall/solar cycle relationships observed at Addis Ababa has applied for the past few hundred years over highland Ethiopia as a whole.

It may be significant that the early 1930s coincided with several changes in climate/solar cycle correlations. From this time the Asmara rainfall shows some solar correlation, and for Addis Ababa, the time lag between rainfall and sunspot peaks decreased. There was, at the same time, a phase change in the relationship between sunspot values and 8 m depth earth temperatures in the UK, and also phase changes in equatorial pressures and temperatures[8]. Additionally, the level of Lake Victoria showed high solar cycle correlation from 1890 to 1930, but the relationship broke down after that time[8]. These changes may be related to the proposed 200 and 700-yr climatic cycles which peaked at that time[1].

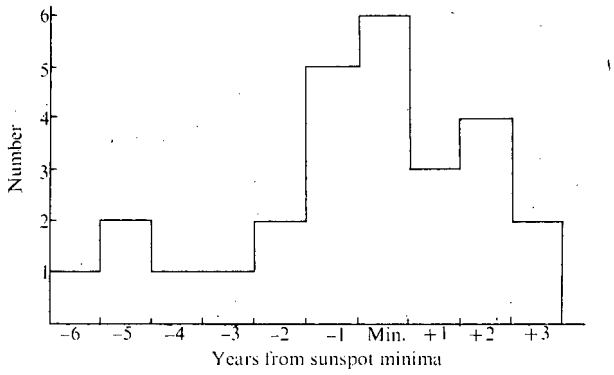

Fig. 3 Distribution of presently known Ethiopian drought years, 1540 to 1974, relative to sunspot minima.

An intensive study of the Indian monsoon rains[9], based upon the 50-yr records of many stations, has found significant correlations with solar cycles. In regions of orographic rainfall near the Himalayas, strong, positive correlations are found, whereas in some other regions there are negative correlations. Zones of positive and negative correlation are separated by areas of little correlation. The Indian data illustrate that the solar influence on rainfall is spatially modulated, in unknown ways, resulting in systematic variations of the sign and degree of correlation. Similar solar cycle influences should be looked for in the rainfall records of the Sahel and elsewhere.

If this sunspot–rainfall relationship is substantiated by

data from other stations in the Sanel and India, governments and relief organisations will have a valuable warning of periods of greatest drought danger.

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Dynamic implications of mantle hotspots

MOVEMENT of lithosphere plates over hotspots which are fixed relative to the mantle could explain the origin of island chains and aseismic ridges[1–4]. Observations of phase velocities against epicentral distances for Tonga–Samoa earthquakes[5] show anomalies which can be interpreted as a radial and lateral inhomogeneity in the lower mantle beneath Hawaii. That indicates the possible existence of deep mantle plumes.

Here, I consider some implications of the mantle plume hypothesis on the basis of a simplified flow model. I represent the flow of the plume by viscous flow through a pipe. The Navier–Stokes equations, assuming steady state, fluid incompressibility and uniform viscosity, reduce to a simple form for the case of pipe flow. The maximum flow velocity, V_z, given by the exact solution of these equations, is

$$V_z = (\mathrm{d}p/\mathrm{d}z)\,(a^2/4\eta) \qquad (1)$$

where a is the radius of the plume, the flow is along z axis and η is the coefficient of viscosity.

$$\text{The volume flux} = (\pi a^2/2)\,V_z \qquad (2)$$

The associated energy equation, based on the same assumptions as for Navier–Stoke equations, also yields an exact solution for pipe flow, giving the temperature δT, generated by the flow:

$$\delta T = \eta\, V_z^2/4K \qquad (3)$$

where K is the coefficient of thermal conductivity.

Table 1 lists the temperature differences and the volume flux for a range of velocities of plume flow.

The values used in computing Table 1 are: $\eta = 3 \times 10^{21}$ g cm^{-1} s^{-1}; $K = 10^{-2}$ calorie °C^{-1} cm^{-1} s^{-1}; and the plume radius, a, = 75 km. The most frequently quoted[2] vertical velocities of plume flow are 2 myr^{-1}. It is clear that such velocities will generate unacceptably high temperatures if the frictional viscosity is of the same order of magnitude as present estimates of the mantle viscosity.

With a speed of flow of 2 m yr^{-1} each plume would bring up about 18 km^3 yr^{-1} of mantle material. Twenty plumes will bring up a total of 360 km^3 yr^{-1}. That would be the total flux of the material moving away from the rise crests. Lithosphere is created at a rate of about 170 km^3 yr^{-1}, which is the flux of counterflow towards the ridge crests. The resultant drag exerted by the flow on the lithosphere will be in the direction of plate motion, that is, away from the rise crests[2]. The drag can be increased by increasing either the number of plumes, or their vertical flow speeds or dimensions.

If, however, the flow speed is reduced to, for instance, 3 cm yr^{-1}, temperatures would become reasonable. But the total flux from 20 plumes would be reduced to about 6 km^3 yr^{-1}. Then, because the total flux of flow towards the rise axes would be many times the flux away from them, the net stress on the plates would tend to close the plates rather than move them apart. Thus, as well as the necessity for a source to provide adequate motive forces for plate motions it becomes necessary to find additional stresses to overcome the closing trend. That adds unnecessary complexity to the problem.

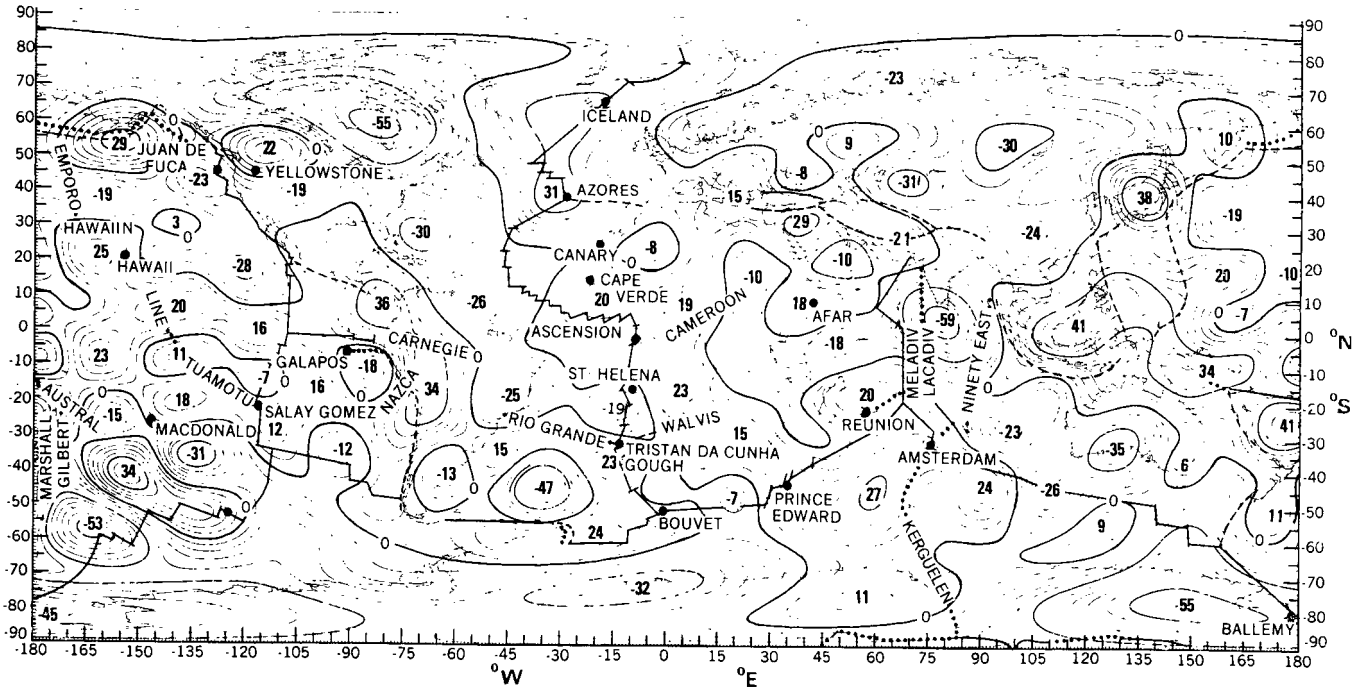

Fig. 1 Relationship between proposed hotspot localities and gravity anomalies (mGal).

Table 1 Typical temperature differences and volume flux for a range of flow velocities

Flow velocity (cm yr^{-1})	Temperature difference (°C)	Volume flux per plume (km^3 yr^{-1})
200	6.75×10^4	18
100	1.69×10^4	9
10	1.69×10^2	9×10^{-1}
3	≈ 15	2.7×10^{-1}
1	≈ 2	9×10^{-2}

Therefore, either an alternative way of keeping down temperatures must be found or the idea that mantle plumes have any significant role in plate tectonics must be rejected.

It may be possible to introduce variations in viscosity and conductivity, although I have assumed uniform viscosity and conductivity. Both, however, are temperature dependent. Therefore, the temperature, δT, generated by the flow, will be affected, but it is uncertain whether the effect would be sufficient to reduce the temperatures to a reasonable level.

Thus, assuming that the results of Table 1 are correct to an order of magnitude then, if the temperatures are to be kept reasonable, some other parameter in equation (3) must be adjusted. The only reasonable possibility at this stage is a reduction in the velocity of the vertical flow of the plume, perhaps accompanied by some increase in its diameter. Unfortunately, that makes the mantle plume hypothesis much less attractive.

It has been contended[2] that the viscous drag exerted on the bottom of the lithosphere by a typical plume, will result in the doming of lithosphere, producing a regional topographic high and a gravity excess over the area. Most hotspots do indeed lie over areas of positive gravity, though very few lie directly over gravity maxima; Fig 1 shows the probable locations of the hotspots, superimposed on isostatic gravity anomalies based on gravity Model GEM 4 (Khan, M. A., and Lerch, F., *et al.*, unpublished). A significant number of hotspots, however, show association with negative gravity; Galapagos, Juan de Fuca, St Helena, Macdonald and Salay Gomez. That contradicts the general claim that plume locations are always associated with positive gravity.

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Λ-type doubling in the CH molecule

ALMOST a year ago we set out to calculate an accurate value of the Λ-doubling in the lowest rotational level of the $X^2\Pi_{\frac{1}{2}}$ state of CH, to assist the radioastronomers, who had been searching for this species in interstellar space for some years. The value we have obtained, which is reported here, represents a substantial improvement on the results of terrestrial experiments[1,2], but its significance is reduced by the astronomical observation of the species, which was reported during the course of this work[3]. The result of the latter experiment indicates just how close our calculation comes to predicting the actual splitting and clearly underlines the power of the methods now available in theoretical chemistry.

Van Vleck[4] has shown that the Λ-doubling of the rotational levels of a $^2\Pi$ state may be represented as a perturbation by $^2\Sigma$ states. To second order, the splitting may be expressed in terms of two parameters, $(\frac{1}{2}p+q)$ and q. For the lowest level of a regular $^2\Pi$ state, for which $J = \Omega = \frac{1}{2}$, the doubling is simply $2(\frac{1}{2}p+q)$, where

$$p = 4\sum \frac{\langle ^2\Pi | H_{so} | ^2\Sigma\rangle \langle ^2\Pi | B(L^+ + L^-) | ^2\Sigma\rangle}{E_\Pi - E_\Sigma}$$

$$\text{and } q = 2\sum \frac{\langle ^2\Pi | B(L^+ + L^-) | ^2\Sigma\rangle^2}{E_\Pi - E_\Sigma}$$

where the summation extends over all vibrational levels of all $^2\Sigma$ states, the contribution from $^2\Sigma^-$ being taken as negative. Matrix elements are taken over both electronic and vibrational wave functions. H_{so} is the spin-orbit coupling operator, and B is the operator $h/8\pi^2\mu r^2$.

An *ab initio* calculation of the parameters p and q was performed for CH by Hinkley *et al.*[5] It was assumed that the electronic matrix elements were independent of internuclear distance, so that vibronic matrix elements could be factorised into electronic and vibrational integrals. The Hartree-Fock orbitals calculated for the $X^2\Pi$ state by Cade and Huo[6] were used; the invarient orbital approximation was made, so that Slater's rules might be applied to the matrix elements. Vibrational wavefunctions were calculated numerically from Morse curves. Only the contributions of the $B^2\Sigma^-$ and $C^2\Sigma^+$ states were considered. The results are shown in Table 1; they are some 20% too low.

In the present work, the calculation of Hinkley *et al.* was modified in several ways. Herzberg and Johns[7] have shown experimentally that both $^2\Sigma$ states have maxima in their potential curves, so that the latter hardly resemble Morse curves. Vibrational wave functions were therefore calculated from the extended configuration interaction potential curves of Lie *et al.*[8], which represent the states rather well. The functions were calculated for the hypothetical case where $J = 0$, and the effects of predissociation on excited vibrational levels were ignored. The vibrational integrals obtained from these wave functions were combined with the electronic matrix elements of Hinkley *et al.* to produce the result labelled (a) in Table 1.

Table 1 Λ-type doubling in the $X^2\Pi_{\frac{1}{2}}$ ($v = 0$, $J = \frac{1}{2}$) state of CH

	$\frac{1}{2}p+q$ (cm^{-1})	q (cm^{-1})	Doubling (MHz)
Hinkley *et al.*[5]	0.0447	0.0254	2,680
This work: (a)	0.0430	0.0245	2,576
(b)	0.0447	0.0274	2,680
(c)	0.0563	0.0386	3,374
(d)	0.0552	0.0379	3,311
Terrestrial experiment[1,2]	0.0563	0.038	3,374 ± 20
Astronomical experiment[3]	0.0556	—	3,335.47 ± 0.01

The error introduced by the invariant orbital approximation was then investigated. A separate set of SCF orbitals was constructed for each of the $^2\Sigma$ states from the basis set of Cade and Huo, using the ALCHEMY program[9]. Electronic matrix elements were then calculated using a program written by J. A. Hall[10]. The values now obtained for the Λ-doubling parameters (b) show that further improvements were necessary. The electronic wave functions were further modified by configuration interaction; six configurations were included for the $X^2\Pi$ and $B^2\Sigma^-$ states, while eight were used for the $C^2\Sigma^+$ state. Electronic matrix elements were again calculated by the Hall method; the result (c) was in close agreement with the experiment.

Finally, the C.I. wave functions were calculated over a range of internuclear distances. The electronic matrix elements were then integrated numerically over the vibrational wave functions. The results (d) are within 1% of the experimental values.

The Λ-doubling is predicted more accurately by this calculation than by terrestrial experimental estimates.

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Drag-reducing polymers and liquid-column oscillations

WE report here on one of many anomalies which occur when drag-reducing polymers are dissolved in water[1–4]: the damping of manometer-type oscillations.

Our apparatus was made from clear PVC tubing (nominal bore 1.3 cm) mounted vertically on a wooden board. The tube was partially filled with liquid, thus creating a rudimentary manometer. The basic experiment consisted of giving the liquid column an initial displacement (25 cm in all cases) from equilibrium and then counting the subsequent oscillations. Graphs of decaying amplitude of oscillation against time were obtained by filming the process at a speed of 18 frames per second. Two pipe configurations were studied: a semicircle, of radius 29 cm; and a conventional U-tube with the bend having a radius of curvature of 4.5 cm. The length of the liquid column (88 cm) was the same in both cases.

In each case the experiment was performed first with water and then with increasing concentrations of drag-reducing polymer in aqueous solution. The additives used were polyethylene oxide (Polyox WSR 301 and WSR N3000) and polyacrylamide (Separan AP 273 and MG 200).

We found that both grades of polyethylene oxide reduced the damping (relative to water) at quite small concentrations; whereas the polyacrylamides, although effective drag-reducers in turbulent flow, gave no measurable reduction.

For small additive concentrations (Polyox WSR 301), a marked reduction in damping (relative to water alone) was evident after the first few oscillations (Fig. 1).

The behaviour at larger concentrations was particularly interesting. For the first few oscillations the damping actually increased (presumably reflecting the increase in solution viscosity) but for the smaller amplitude motions a reduction in damping again occurred (Fig. 1). These results suggest that, during the first few oscillations, the flow is controlled by the viscosity of the solution alone.

The corresponding results for the U-tube were very similar; there is little apparent difference between the two flow configurations (Fig. 1).

Counting the total number of oscillations does, however, reveal that the additives have surprising quantitative effects on the damping. If the total number of oscillations with water is N_w and with polymer solution N_p, the percentage increase in the number of oscillations can be defined as

$$100\ (N_p - N_w)/N_w$$

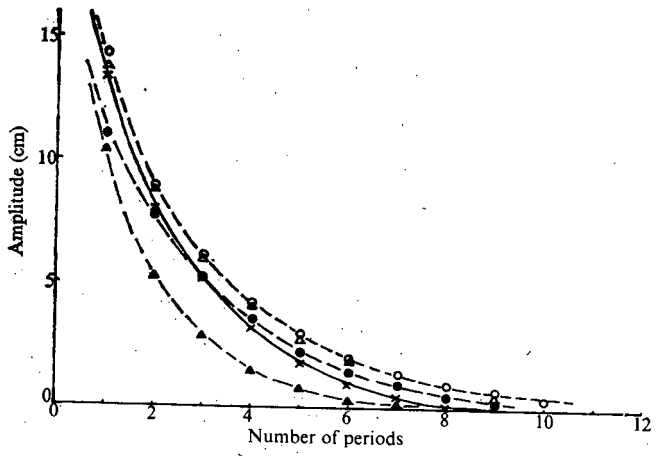

Fig. 1 Amplitude of oscillation against time for Polyox WSR 301 in a semicircular tube. ×, Water; ○, 10 p.p.m. WSR 301; △, 25 p.p.m. WSR 301; ●, 250 p.p.m. WSR 301; ▲, 500 p.p.m WSR 301. Each period = 1.6 s.

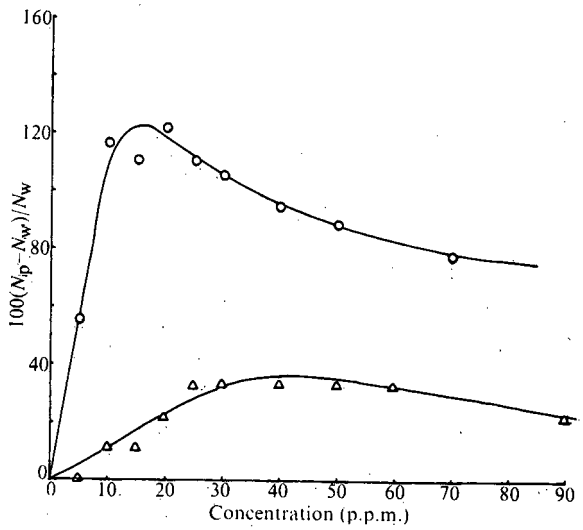

Fig. 2 Variation of damping reduction (assessed by observing the percentage increase in the total number of oscillations) with concentration of Polyox WSR 301. ○, Semicircular tube; △, U-tube.

Results for the semicircular tube (Fig. 2) show that the total number of oscillations is more than doubled in the concentration range 10–30 p.p.m. On the other hand, the U-tube (Fig. 2) shows a much smaller peak enhancement of about 30–40%, over the broader range of 25–65 p.p.m.

Polyethylene oxide tends to adhere to the pipe wall. In an attempt to assess the importance of this wall coating, a tube which had previously been used with solution concentrations of up to 500 p.p.m. was rinsed out, to remove any remaining drops of polymer solution and the tests were then repeated, beginning with water. It was found that, when water was added to a coated tube, there was a considerable degree of enhancement (relative to water in a clean tube). To exclude the unlikely possibility that a portion of the polymer coating had dissolved in the fresh

water, the sample was decanted after a marked reduction had been observed. It was then placed in a clean PVC tube and the experiment was repeated and no significant reduction in damping was observed. It is, therefore, difficult to avoid the conclusion that observed reductions in damping occur because of an adsorbed layer of polymer additive on the tube wall, rather than because of the viscoelastic properties of the actual solution.

Our results indicate that very small amounts of polyethylene oxide can produce quite large reductions in the degree of damping of manometer-type oscillations. The failure of polyacrylamide to show any enhancement may result from the fact that it does not seem to coat the tube, or it may conceivably be a matter of time scales. Unfortunately, as the period of oscillation is proportional to the square root of the length of liquid in the tube, an investigation of much smaller time scales would have its difficulties.

The much greater reduction in the degree of damping found with the semicircular tube is presumably because the tube is entirely curved, whereas the U-tube is only partially so. This would be consistent with previous results[5] for steady flow in curved pipes, in which polymer additives resulted in marked reductions in drag.

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1/*f* noise with a low frequency white noise limit

One of us (A.A.V.), who had recently been studying channel models for electrical fluctuations caused by potassium transport through nerve membrane, noticed a possible analogy with the flow pattern of grain through an hourglass of unusual shape designed by F. Vaudan, Paris. The hourglass was filled with small grains (0.1 to 1.0 mm diameter) of coloured glass and differed from ordinary hourglasses in the length of the pore connecting the two compartments which was drawn out to about 25 cm; about 80 times the smallest inner diameter (3 mm). When the hourglass was turned, the grain did not flow steadily but exhibited slow irregular density fluctuations (Fig. 1). With a view to the analogy with potassium channels in excitable membranes, we decided to examine comparative power spectra[1-3].

In a series of experiments to measure spectral properties of the grain flow, a laser beam (Spectra-Physics model 155) was sent through the stem of the hourlgass and then through a small hole (0.2 mm diameter) placed in front of and close to a photosensitive cell and a dc amplifier.

Light-beam switching resulted in square-waves with a resolution time of about 10^{-4} seconds for the system of cell and amplifier. Another test using a white-noise modulated light source with a white band between 1 and 5,000 Hz showed that the system response was flat within the available range.

In the resulting pulse sequence, most of the pulses had comparable shapes (about triangular, or triangular with clipped peaks when the grains completely blocked the beam during part of their passage) and durations which varied from about 10^{-3} s to 10^{-2} s. After amplification, the power spectrum was calculated (Fig. 2, curve A). Its shape did not change for measurements through different levels of the stem (higher up, where the stream is dense and the light shines through occasionally, or lower down, where individual particles pass through the beam).

The mechanism responsible for the spectral shape must be the interaction between particles and wall and between the particles themselves, while a third friction coefficient is given by the counterflow of air. Clustering is known to occur in bins in the absence of a counterflow of air. The actual values of the friction coefficients (between particles and wall and between the particles themselves) are not of primary importance[4], although they may influence the detailed structure of the spectrum, such as the corner frequencies and the actual slope of the $1/f$ part.

We therefore also examined the spectrum associated with free fall flow out of an open compartment through a gradually narrowing pore (3 mm final diameter) (Fig. 2, curve B). Irregularly shaped, sharp edged steel grit (0.2 mm mean diameter) was used. In both systems the spectrum was white at low frequencies and changed to $1/f$ at higher frequencies. At still higher frequencies the spectrum shape is sensitive to several system parameters and is rather complex.

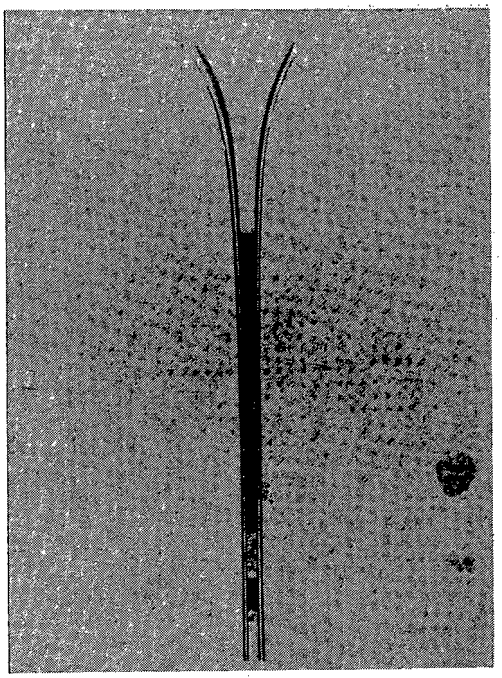

Fig. 1 Flow through hourglass exhibiting clustering (exposure time 1/1000 s).

This is to our knowledge the first report on a physical system with $1/f$ noise which changes at lower frequencies into a different and finite power spectrum. No special tricks need to be invoked, therefore, to circumvent the famous $1/f$ paradox with its explosion of power for frequencies approaching zero[5]. The mechanism at work here might, for this reason, be of general interest, since noise power spectra are investigated in a diversity of fields such as magnetism, superconductivity, semiconductors, artificial and biological membranes and synapses.

Furthermore, the transition from a $1/f$ spectral density into some other spectral density is not the kind predicted by McWhorter[6]; there one must invoke a Poisson process and a distribution of pulse time constants.

It is not very difficult to explain some of the major features of the measured spectra[7]. These include the low frequency form of the power spectrum (white) and the bandwidth over which the spectrum is essentially $1/f$. Further, it is possible to show, in general, the conditions under which one may expect deviations from a white spectrum and from this obtain important additional insights into possible source mechanisms.

In order to demonstrate these ideas, let us[7], following Heiden[8], view a noise sequence as a sequence of unitary events (pulses) which may represent current, voltage, magnetic lines, and so on. Each individual pulse has its own time constant, τ, amplitude, h, and shape $y(t,\tau)$. In addition, characterising

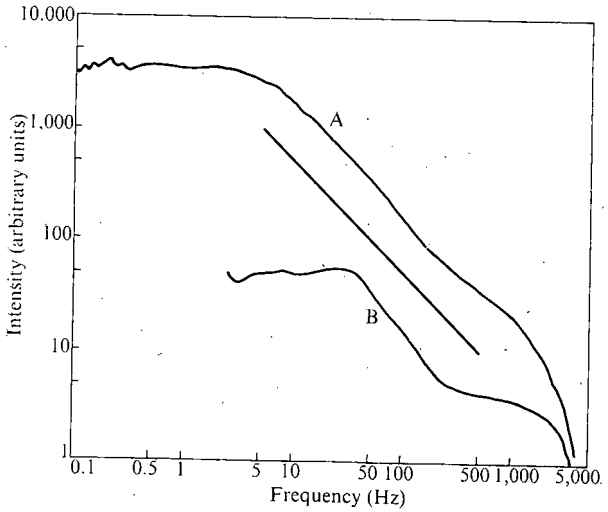

Fig. 2 Power spectra associated with particle flow through hourglass (curve A) and through a narrow pore out of an open compartment (curve B). Line between curves indicates slope of (ideal) $1/f$ noise.

each pulse is the interval φ between its beginning and the beginning of the next pulse. The simplified sequence can be drawn as in Fig. 3.

The interval distribution function $\mu(\varphi)$ may be Poisson or non-Poisson. The sequence can be written as

$$I(t) = h_1 y(t, \tau_1) + h_2 y(t - \varphi_1, \tau_2) + h_3 y(t - \varphi_1 - \varphi_2, \tau_3) + \ldots$$

For those sequences in which φ, τ, and h are functionally independent of one another, the power spectrum is[7]

$$S(f) = N\langle h^2\rangle\langle|F(f,\tau)|\rangle^2\left[1+\frac{2\langle h\rangle^2}{\langle h^2\rangle}\frac{|\langle F(f,\tau)\rangle|^2}{\langle|F(f,\tau)|\rangle^2}\mathrm{Re}\left(\frac{\psi}{1-\psi}\right)\right]$$

where $\langle\ \rangle$ indicates average value, N is the mean number of pulses per unit time, $F(f,\tau)$ is the Fourier transform of the individual pulse form $y(t,\tau)$, and

$$\psi = \int_0^\infty \mu(\varphi)\exp(2\pi i f\varphi)\mathrm{d}\varphi.$$

For convenience, we can write $S(f) = NA(f)P(f)$ where $A(f) = \langle h^2\rangle\langle|F(f,\tau)|\rangle^2$ and $P(f)$ is the expression in the brackets.

For Poisson distributions, $\mu(\varphi) = N\exp(-N\varphi)$ and Re $(\psi/(1-\psi))$ is zero. In these cases, then, the form of the power spectrum is determined by $F(f,\tau)$ and the distribution of pulse time constants, $\nu(\tau)$.

In general,

$$F(f,\tau) = \int_{-\infty}^{+\infty} y(t,\tau)\exp(-2\pi i f t)\,\mathrm{d}t.$$

At low frequencies ($2\pi f\tau \ll 1$), however,

$$F(f,\tau) \cong \int_{-\infty}^{+\infty} y(t,\tau)\mathrm{d}t.$$

Hence the Fourier transform is frequency independent and the low frequency portion of the spectrum is white[9].

If there is a distribution of time constants, $\nu(\tau)$, among the pulses, then, physically, there exists a maximum τ, τ_m, such that $\nu(\tau)$ is small for $\tau > \tau_m$. Now

$$\langle F(f,\tau)\rangle \simeq \int_0^{\tau_m} F(f,\tau)\nu(\tau)\mathrm{d}\tau,$$

so at frequencies ($2\pi f\tau_m \ll 1$), $\langle F(f,\tau)\rangle$ is also frequency independent and represents a white spectrum. This is also true for $\langle|F(f,\tau)|\rangle$. Hence, for a sequence of pulses with a distribution of time constants, $A(f)$ is white at low enough frequencies so long as there is no coupling among the parameters of a single pulse.

A similar conclusion obtains for the factor $P(f)$ which incorporates the effect on the power spectrum of deviations from a Poisson sequence.

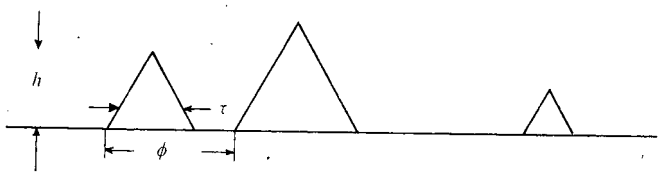

Fig. 3 Pulse sequence parameters.

We have

$$\psi = \int_0^\infty \mu(\varphi)\exp(2\pi i f\varphi)\,\mathrm{d}\varphi.$$

Physically there exists a maximum φ, say φ_m, beyond which $\mu(\varphi)$ is arbitrarily small. Hence, for low enough frequencies, ($2\pi f\varphi_m \ll 1$), ψ is frequency independent and, therefore, so is Re $(\psi/(1-\psi))$.

We therefore have a theorem which says that the power spectrum of any pulse sequence, Poisson or non-Poisson, even with a distribution of pulse time constants, is white at low enough frequencies provided that there is no coupling among the parameters of a single pulse.

There are at least two immediate and useful consequences. First, if there is not too much overlap between pulses, simple visual inspection of the sequence will permit estimates of τ_m and φ_m. This in turn allows an approximate determination of the low frequency bandwidths over which $A(f)$ and $P(f)$ respectively are white. Hence, under these conditions, visual inspection can tell us whether deviations from a white spectrum are due to $A(f)$ or $P(f)$ over a particular bandwidth. This is particularly easy to do in those cases where $\varphi_m \gg \tau_m$. Secondly, if one should find that at low frequencies ($2\pi f \ll$ the smaller of τ_m^{-1} or φ_m^{-1}) the spectrum is not white, then the implication is that coupling must exist among the parameters of a single pulse.

The first consequence permits direct comparison between the experimentally determined form of the power spectrum and the theoretical frequency range over which $A(f)$ and $P(f)$ should be white. Since one now knows over what bandwidth the form of the spectrum is due to the shape of the individual pulses, we have a direct way of determining if the sequence is non-Poisson (via $P(f)$). The second allows one to deduce that functional relations (for example, φ = (constant)τ or h = constant/φ) exist between the pulse parameters. We note for example that φ = (constant)τ is a kind of inhibition where the presence of a pulse with a long time constant tends to delay the appearance of a next pulse while h = constant/φ is a kind of facilitation where a large pulse tends to encourage the appearance of the subsequent pulse. Detailed analysis of the low frequency deviations from a white spectrum will make it possible, in some cases, to select the precise form of the pulse coupling and thereby give further insight into the physical origins of the noise.

If the hourglass flow is examined at different angles with respect to the vertical, the formation of unstable vaults can easily be seen. Clearly, the formation of unstable vaults of different lifetimes generates the clustering effects (Fig. 1) in the flow. In an extensive study (published in Dutch) Peschl[4] investigated the flow of particles from bin openings. A key parameter is the ratio of the opening to the grain diameter. In an hourglass the ratio threshold, 4, is exceeded.

Only unstable vaults occur in normal use and their lifetimes have an upper limit. Hence, the distribution of the intervals between successive particles flowing through a plane must have an upper time limit. In the case of the flow of steel grit through a pore, in our experiments, for example, no intervals longer than 200 ms were found during a period of 10 min with a mean flow of 1,250 grains s^{-1} through the laser beam (0.2 mm diameter). Generally, the individual pulse time constants were considerably less than 20 ms. According to the theorem developed earlier in the paper, the low frequency spectrum should be white for frequencies below about $1/0.2 = 5$ Hz as has been found (Fig. 2). The $1/f$ portion of the spectrum is due to the non-Poisson character (clustering) of the individual particles. At higher

frequencies a change from $1/f$ to some other spectrum is predicted due to pulse shape, time constant and photocell response. This occurs, as expected, at frequencies $\gg 100$ Hz.

It is tempting to conjecture that in some systems of molecular dimensions with barriers, particles and pores analogous situations might prevail. Molecular size vaults might be formed and thermal motion function as the agent for the introduction of the instabilities. These systems would exhibit power spectra with strong low frequency contributions (perhaps $1/f$ in form) but, yet, at the very lowest frequencies their spectra would always turn white.

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Second Law of Thermodynamics

IT is widely realised that there is no single and uniquely correct statement of the Second Law of Thermodynamics but rather that there exist a number of different and mutually compatible, correct statements[1] (Everett[2] mentions "two or three dozen") each of which illuminates a different facet of what H. A. Bent (private communication) has called the "Second Law type of behaviour observed in nature". There is no brief statement from which " . . . all of the thermodynamic relationships . . ."[3] can be deduced without further knowledge. In considering a particular problem one particular statement is generally more apposite than another, and for those who are concerned with the mechanisms that produce work, a statement of the Law that stresses the significance of work has distinct advantages. One such statement of mine[4] has been criticised by Legon[3]. Unfortunately the revised article containing the basis for some of my statements has only recently appeared[5] though it was submitted long before the other[4]. This paper[5] should have forestalled many of Legon's objections. For example, its first page deals with the question of "maximum work" and its equations (5) to (7) and (9) to (13) deal with equilibria and with entropy creation respectively.

Legon's objections are numerous and diverse but I will try to deal systematically with them, as summarised in his last paragraph.

Lack of originality. I had not seen the passage quoted from Butler[6] (page 32) when I wrote my paper[4]. Legon writes of my statement: "All it says is that something that is happening" (that is, that can proceed spontaneously) "can be made to do useful work". Exactly so—it is very simple. Taken with its corollary, that unless a proposed change can be made to do useful work it cannot happen spontaneously, the statement specifies conditions both necessary and sufficient for predicting which changes can happen spontaneously and which cannot. There is no logical necessity to add any statement about "maximum work". Readers of my two articles[4,5] can surely be in little doubt that I understand at least as well as does Legon the fundamental conclusion of Carnot[7] that to every specified change (isothermal or not) there must correspond a certain definite maximum output of work when going in one direction and an equally definite minimum input of work when going in the opposite one: these transfers of work would be realised under reversible conditions; they may or may not be equal depending on the particular system under consideration.

I made no claim that my statement of the Second Law sums up the whole of thermodynamics in one sentence: neither for that matter does Legon's italicised quotation from Planck[8] (page 103) do so. It is necessary to know more of the subject in order to comprehend either statement, and the two can then be seen as not contradictory, but complementary.

To understand the 'work' view of the subject in relation to the 'entropy' view of it, one must understand the fact (explicitly stated in refs 4 and 5) that in order to obtain work from a spontaneous process a machine of appropriate design must be introduced, in imagination at least, into the situation. This machine constrains the process in such a way that it can occur only if at the same time work is delivered; the machine must also contain a device (for example, a weight) for storing this work since many types of system are not, in themselves, capable of storing work. All machines consist of several phases and possess geometrical features, and far from being a mere artifice, the nature of such machines and their limitations are the main topics of interest that thermodynamics has had to offer to many scientists, from the Carnots onwards. As Joule[9] (who was interested in muscles as well as in galvanic cells and heat engines) wrote in 1853: "Perhaps the most important applications of dynamical theory are those which refer to the production of motive power from chemical and other actions." The matter could not be more succinctly expressed.

Table 1 Range of spontaneous processes

Completely irreversible ..... **Completely reversible**
Resulting change in Universe
Maximum entropy creation ..... Zero entropy creation
Zero increase in stored work ..... Max. increase in stored work
Max. inc. in thermal energy ..... Min. inc. in thermal energy

Failure to apply to isolated systems. I did not in fact claim[4] that the same equation relating work to entropy creation applied to isolated systems, merely that the "argument can be easily extended" to them, by imagining a machine to be introduced into the system itself. This basic idea is far from novel[10,11]. A simple example that illustrates the point well is provided by an isolated system in two parts that are initially at different temperatures. Heat is allowed to flow either by ordinary conduction or through a heat engine. For a given change of state it is found that the entropy created ΔS_{cr} is always a single-valued function of the work wasted. If one part of the system (e) is much larger than the other the equations approach as a limit the simple form given in my paper (ref. 5, equation (10)):

$$\Delta S_{cr} = [\text{wasted work}]/T_e$$

Legon's preoccupation with semantics has led him to make heavy weather out of a simple situation. Work can certainly be stored in an isolated system—by lifting a weight within the system or in various other ways. Revealingly enough, the one form of energy that cannot be used for this purpose is the very one suggested by Legon, that is, thermal energy. The fundamental distinction between thermal energy (for definition, see ref. 12, page 11) and other types of energy is absolutely basic.

Legon poses, as a challenge, the problem of obtaining work from the mixing of two ideal gases in an isolated system of constant total volume. It is elementary that if the mixture is allowed to form by merely withdrawing a partition between the gases we have a good example of a completely irreversible process with maximal entropy creation ($+11.53$ J K^{-1} if we started with 1 mol of each at 300 K) and no performance or storage of work. On the other hand, by introducing into the system a suitable machine, the uniform mixture could be allowed to form in such a way that a weight within the system was raised. (The machine described by Planck (ref. 8, page 219) may be readily adapted for this purpose.) At the end of the latter mixing process the isolated system would accordingly contain more mechanical energy than it did at the beginning. From the First Law it follows that the system must necessarily contain less thermal energy; that is, its temperature must have fallen. In the limit, where the mixing was reversible, the maximum possible work would have been performed and transferred to the weight (2,769 J if the gases were monatomic) and the temperature would have fallen to 189 K. In this reversible case the change in entropy arising from mixing ($+11.53$ J K^{-1}) is exactly counterbalanced by that attributable to cooling (-11.53 J K^{-1}): no entropy is created.

At this point it might be objected that the change in the gases is not exactly the same as if they had mixed irreversibly, because their thermal energy and temperature have decreased. This is a simple consequence of the First Law which applies equally no matter whether one is considering an isolated system, a non-isolated one or the whole Universe. If a change is conducted in such a way that a weight is lifted then all the other bodies involved cannot possibly end up in the same state as if the weight had not been lifted.

Failure to apply to nonisothermal systems. Legon expresses doubts about the validity of the equation for entropy creation (refs 3 and 4) save for "the trivial case for which the temperature T_e of the environment is equal to the temperature T of the system throughout the process"[3]. On what grounds are these doubts based? Legon does not discuss, let alone dismiss, any of the sources quoted in my article[5]. Other relevant sources which should be considered are Keenan and Hatsopoulos[13] and the classic accounts by Maxwell[10] and by Gouy[14].

Legon's quotation from Planck (ref. 8, page 104) concerning "dissipated energy" deserves close consideration. It seems to state that the maximum work is a definite quantity only for isothermal processes. If true this would directly contradict the views of Thomson[15] (later Lord Kelvin) "On a universal tendency in Nature to the dissipation of mechanical energy". On pages 113–117 of ref. 8, however, Planck discusses his own statement (ref. 8, page 104) and we see that there is in fact no contradiction. What Planck demonstrates is that although the change in Helmholtz free energy, $-dA = -d(U-TS)$, measures w_{max} under isothermal conditions, it cannot conveniently be used to determine w_{max} under nonisothermal conditions because the term $S\,dT$ that then appears is frequently indeterminate. The same point has already been made in a footnote by Gouy (ref. 15, page 506) who had also given the correct equation for determining w_{max} under nonisothermal conditions. Accordingly I find no substance in Legon's objections under this heading.

If it is thought that there is conflict between the 'work' view of thermodynamics and the 'entropy' view it is high time that the idea was abandoned. The two views are different, but symmetrical, aspects of the same reality. Spontaneous processes of all kinds fall somewhere within the pattern shown in Table 1, their position depending on the efficiency of the machinery used for the extraction of work.

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Information transmission under conditions of sensory shielding

WE present results of experiments suggesting the existence of one or more perceptual modalities through which individuals obtain information about their environment, although this information is not presented to any known sense. The literature[1-3] and our observations lead us to conclude that such abilities can be studied under laboratory conditions.

We have investigated the ability of certain people to describe graphical material or remote scenes shielded against ordinary perception. In addition, we performed pilot studies to determine if electroencephalographic (EEG) recordings might indicate perception of remote happenings even in the absence of correct overt responses.

We concentrated on what we consider to be our primary responsibility—to resolve under conditions as unambiguous as possible the basic issue of whether a certain class of paranormal perception phenomena exists. So we conducted our experiments with sufficient control, utilising visual, acoustic and electrical shielding, to ensure that all conventional paths of sensory input were blocked. At all times we took measures to prevent sensory leakage and to prevent deception, whether intentional or unintentional.

Our goal is not just to catalogue interesting events, but to uncover patterns of cause–effect relationships that lend themselves to analysis and hypothesis in the forms with which we are familiar in scientific study. The results presented here constitute a first step towards that goal; we have established under known conditions a data base from which departures as a function of physical and psychological variables can be studied in future work.

REMOTE PERCEPTION OF GRAPHIC MATERIAL

First, we conducted experiments with Mr Uri Geller in which we examined his ability, while located in an electrically shielded room, to reproduce target pictures drawn by experimenters located at remote locations. Second, we conducted double-blind experiments with Mr Pat Price, in which we measured his ability to describe remote outdoor scenes many miles from his physical location. Finally, we conducted pre-

liminary tests using EEGs, in which subjects were asked to perceive whether a remote light was flashing, and to determine whether a subject could perceive the presence of the light, even if only at a noncognitive level of awareness.

In preliminary testing Geller apparently demonstrated an ability to reproduce simple pictures (line drawings) which had been drawn and placed in opaque sealed envelopes which he was not permitted to handle. But since each of the targets was known to at least one experimenter in the room with Geller, it was not possible on the basis of the preliminary testing to discriminate between Geller's direct perception of envelope contents and perception through some mechanism involving the experimenters, whether paranormal or subliminal.

So we examined the phenomenon under conditions designed to eliminate all conventional information channels, overt or subliminal. Geller was separated from both the target material and anyone knowledgeable of the material, as in the experiments of ref. 4.

In the first part of the study a series of 13 separate drawing experiments were carried out over 7 days. No experiments are deleted from the results presented here.

At the beginning of the experiment either Geller or the experimenters entered a shielded room so that from that time forward Geller was at all times visually, acoustically and electrically shielded from personnel and material at the target location. Only following Geller's isolation from the experimenters was a target chosen and drawn, a procedure designed to eliminate pre-experiment cueing. Furthermore, to eliminate the possibility of pre-experiment target forcing, Geller was kept ignorant as to the identity of the person selecting the target and as to the method of target selection. This was accomplished by the use of three different techniques: (1) pseudo-random technique of opening a dictionary arbitrarily and choosing the first word that could be drawn (Experiments 1–4); (2) targets, blind to experimenters and subject, prepared independently by SRI scientists outside the experimental group (following Geller's isolation) and provided to the experimenters during the course of the experiment (Experiments 5–7, 11–13); and (3) arbitrary selection from a target pool decided upon in advance of daily experimentation and designed to provide data concerning information content for use in testing specific hypotheses (Experiments 8–10). Geller's task was to reproduce with pen on paper the line drawing generated at the target location. Following a period of effort ranging from a few minutes to half an hour, Geller either passed (when he did not feel confident) or indicated he was ready to submit a drawing to the experimenters, in which case the drawing was collected before Geller was permitted to see the target.

To prevent sensory cueing of the target information, Experiments 1 through 10 were carried out using a shielded room in SRI's facility for EEG research. The acoustic and visual isolation is provided by a double-walled steel room, locked by means of an inner and outer door, each of which is secured with a refrigerator-type locking mechanism. Following target selection when Geller was inside the room, a one-way audio monitor, operating only from the inside to the outside, was activated to monitor Geller during his efforts. The target picture was never discussed by the experimenters after the picture was drawn and brought near the shielded room. In our detailed examination of the shielded room and the protocol used in these experiments, no sensory leakage has been found.

The conditions and results for the 10 experiments carried out in the shielded room are displayed in Table 1 and Fig. 1. All experiments except 4 and 5, were conducted with Geller inside the shielded room. In Experiments 4 and 5, the procedure was reversed. For those experiments in which Geller was inside the shielded room, the target location was in an adjacent room at a distance of about 4 m, except for Experiments 3 and 8, in which the target locations were, respectively, an office at a distance of 475 m and a room at a distance of about 7 m.

A response was obtained in all experiments except Numbers 5–7. In Experiment 5, the person-to-person link was eliminated by arranging for a scientist outside the usual experimental group to draw a picture, lock it in the shielded room before Geller's arrival at SRI, and leave the area. Geller was then led

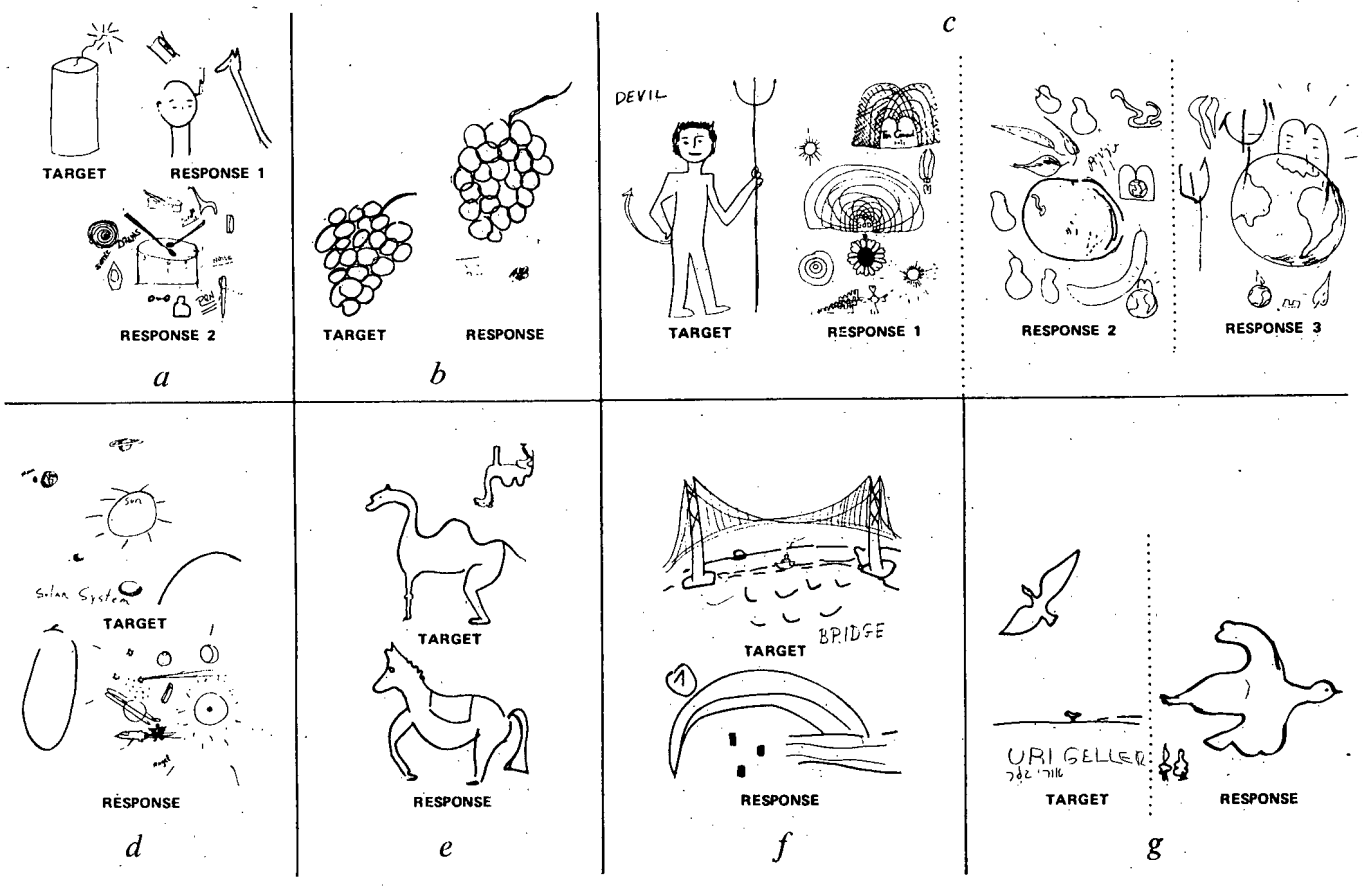

Fig. 1 Target pictures and responses drawn by Uri Geller under shielded conditions.

Table 1 Remote perception of graphic material

Experiment	Date (month, day, year)	Geller Location	Target Location	Target	Figure
1	8/4/73	Shielded room 1*	Adjacent room (4.1 m)†	Firecracker	1*a*
2	8/4/73	Shielded room 1	Adjacent room (4.1 m)	Grapes	1*b*
3	8/5/73	Shielded room 1	Office (475 m)	Devil	1*c*
4	8/5/73	Room adjacent to shielded room 1	Shielded room 1 (3.2 m)	Solar system	1*d*
5	8/6/73	Room adjacent to shielded room 1	Shielded room 1 (3.2 m)	Rabbit	No drawing
6	8/7/73	Shielded room 1	Adjacent room (4.1 m)	Tree	No drawing
7	8/7/73	Shielded room 1	Adjacent room (4.1 m)	Envelope	No drawing
8	8/8/73	Shielded room 1	Remote room (6.75 m)	Camel	1*e*
9	8/8/73	Shielded room 1	Adjacent room (4.1 m)	Bridge	1*f*
10	8/8/73	Shielded room 1	Adjacent room (4.1 m)	Seagull	1*g*
11	8/9/73	Shielded room 2‡	Computer (54 m)	Kite (computer CRT)	2*a*
12	8/10/73	Shielded room 2	Computer (54 m)	Church (computer memory)	2*b*
13	8/10/73	Shielded room 2	Computer (54 m)	Arrow through heart (computer CRT, zero intensity)	2*c*

*EEG Facility shielded room (see text).
†Perceiver–target distances measured in metres.
‡SRI Radio Systems Laboratory shielded room (see text).

by the experimenters to the shielded room and asked to draw the picture located inside the room. He said that he got no clear impression and therefore did not submit a drawing. The elimination of the person-to-person link was examined further in the second series of experiments with this subject.

Experiments 6 and 7 were carried out while we attempted to record Geller's EEG during his efforts to perceive the target pictures. The target pictures were, respectively, a tree and an envelope. He found it difficult to hold adequately still for good EEG records, said that he experienced difficulty in getting impressions of the targets and again submitted no drawings.

Experiments 11 through 13 were carried out in SRI's Engineering Building, to make use of the computer facilities available there. For these experimenters, Geller was secured in a double-walled, copper-screen Faraday cage 54 m down the hall and around the corner from the computer room. The Faraday cage provides 120 dB attenuation for plane wave radio frequency radiation over a range of 15 kHz to 1 GHz. For magnetic fields the attenuation is 68 dB at 15 kHz and decreases to 3 dB at 60 Hz. Following Geller's isolation, the targets for these experiments were chosen by computer laboratory personnel not otherwise associated with either the experiment or Geller, and the experimenters and subject were kept blind as to the contents of the target pool.

For Experiment 11, a picture of a kite was drawn on the face of a cathode ray tube display screen, driven by the computer's graphics program. For Experiment 12, a picture of a church was drawn and stored in the memory of the computer. In Experiment 13, the target drawing, an arrow through a heart (Fig. 2*c*), was drawn on the face of the cathode ray tube and then the display intensity was turned off so that no picture was visible.

To obtain an independent evaluation of the correlation between target and response data, the experimenters submitted the data for judging on a 'blind' basis by two SRI scientists who were not otherwise associated with the research. For the 10 cases in which Geller provided a response, the judges were asked to match the response data with the corresponding target data (without replacement). In those cases in which Geller made more than one drawing as his response to the target, all the drawings were combined as a set for judging. The two judges each matched the target data to the response data with no error. For either judge such a correspondence has an *a priori* probability, under the null hypothesis of no information channel, of $P = (10!)^{-1} = 3 \times 10^{-7}$.

A second series of experiments was carried out to determine whether direct perception of envelope contents was possible without some person knowing of the target picture.

One hundred target pictures of everyday objects were drawn by an SRI artist and sealed by other SRI personnel in double envelopes containing black cardboard. The hundred targets were divided randomly into groups of 20 for use in each of the three days' experiments.

On each of the three days of these experiments, Geller passed. That is, he declined to associate any envelope with a drawing that he made, expressing dissatisfaction with the existence of such a large target pool. On each day he made approximately 12 recognisable drawings, which he felt were associated with the entire target pool of 100. On each of the three days, two of his drawings could reasonably be associated with two of the 20 daily targets. On the third day, two of his drawings were very close replications of two of that day's target pictures. The drawings resulting from this experiment do not depart significantly from what would be expected by chance.

In a simpler experiment Geller was successful in obtaining information under conditions in which no persons were knowledgeable of the target. A double-blind experiment was performed in which a single 3/4 inch die was placed in a $3 \times 4 \times 5$ inch steel box. The box was then vigorously shaken by one of the experimenters and placed on the table, a technique found in control runs to produce a distribution of die faces differing non-significantly from chance. The orientation of the die within the box was unknown to the experimenters at that time. Geller would then write down which die face was uppermost. The target pool was known, but the targets were individually prepared in a manner blind to all persons involved in the experiment. This experiment was performed ten times, with Geller passing twice and giving a response eight times. In the eight times in which he gave a response, he was correct each time. The distribution of responses consisted of three 2s, one 4, two 5s, and two 6s. The probability of this occurring by chance is approximately one in 10^6.

In certain situations significant information transmission can take place under shielded conditions. Factors which appear to be important and therefore candidates for future investigation include whether the subject knows the set of targets in the target pool, the actual number of targets in the target pool at any given time, and whether the target is known by any of the experimenters.

It has been widely reported that Geller has demonstrated the ability to bend metal by paranormal means. Although metal bending by Geller has been observed in our laboratory, we have not been able to combine such observations with adequately controlled experiments to obtain data sufficient to support the paranormal hypothesis.

REMOTE VIEWING OF NATURAL TARGETS

A study by Osis[5] led us to determine whether a subject could describe randomly chosen geographical sites located several miles from the subject's position and demarcated by some

appropriate means (remote viewing). This experiment carried out with Price, a former California police commissioner and city councilman, consisted of a series of double-blind, demonstration-of-ability tests involving local targets in the San Francisco Bay area which could be documented by several independent judges. We planned the experiment considering that natural geographical places or man-made sites that have existed for a long time are more potent targets for paranormal perception experiments than are artificial targets prepared in the laboratory. This is based on subject opinions that the use of artificial targets involves a 'trivialisation of the ability' as compared with natural pre-existing targets.

In each of nine experiments involving Price as subject and SRI experimenters as a target demarcation team, a remote location was chosen in a double-blind protocol. Price, who remained at SRI, was asked to describe this remote location, as well as whatever activities might be going on there.

Several descriptions yielded significantly correct data pertaining to and descriptive of the target location.

In the experiments a set of twelve target locations clearly differentiated from each other and within 30 min driving time from SRI had been chosen from a target-rich environment (more than 100 targets of the type used in the experimental series) prior to the experimental series by an individual in SRI management, the director of the Information Science and Engineering Division, not otherwise associated with the experiment. Both the experimenters and the subject were kept blind as to the contents of the target pool, which were used without replacement.

An experimenter was closeted with Price at SRI to wait 30 min to begin the narrative description of the remote location. The SRI locations from which the subject viewed the remote locations consisted of an outdoor park (Experiments 1, 2), the double-walled copper-screen Faraday cage discussed earlier (Experiments 3, 4, and 6–9), and an office (Experiment 5). A second experimenter would then obtain a target location from the Division Director from a set of travelling orders previously prepared and randomised by the Director and kept under his control. The target demarcation team (two to four SRI experimenters) then proceeded directly to the target by automobile without communicating with the subject or experimenter remaining behind. Since the experimenter remaining with the subject at SRI was in ignorance both as to the particular target and as to the target pool, he was free to question Price to clarify his descriptions. The demarcation team then remained at the target site for 30 min after the 30 min allotted for travel. During the observation period, the remote-viewing subject would describe his impressions of the target site into a tape recorder. A comparison was then made when the demarcation team returned.

Price's ability to describe correctly buildings, docks, roads, gardens and so on, including structural materials, colour, ambience and activity, sometimes in great detail, indicated the functioning of a remote perceptual ability. But the descriptions contained inaccuracies as well as correct statements. To obtain a numerical evaluation of the accuracy of the remote viewing experiment, the experimental results were subjected to independent judging on a blind basis by five SRI scientists who were

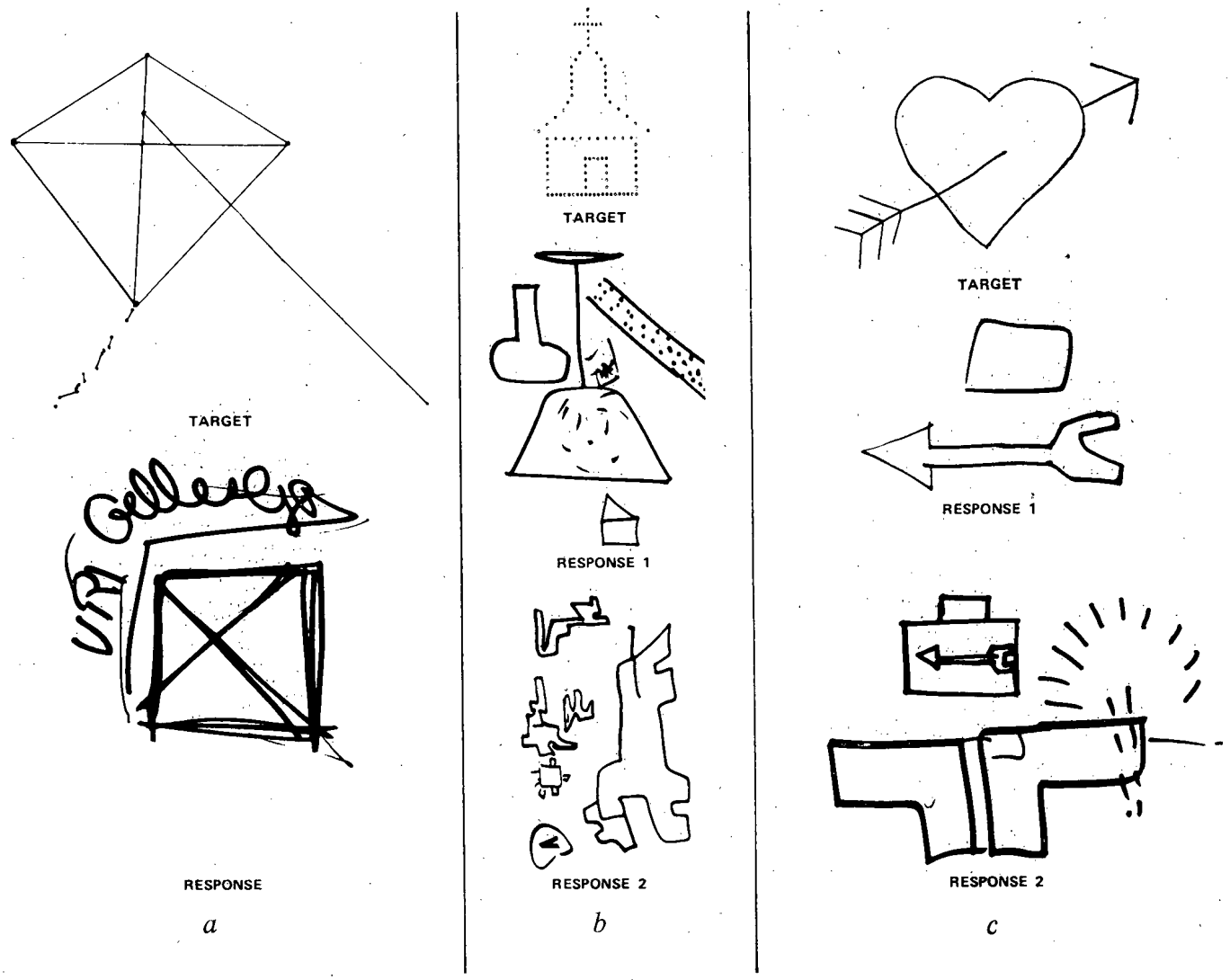

Fig. 2 Computer drawings and responses drawn by Uri Geller. *a*, Computer drawing stored on video display; *b*, computer drawing stored in computer memory only; *c*, computer drawing stored on video display with zero intensity.

Table 2 Distribution of correct selections by judges A, B, C, D, and E in remote viewing experiments

Descriptions chosen by judges		Places visited by judges								
		1	2	3	4	5	6	7	8	9
Hoover Tower	1	**ABCDE**				D				
Baylands Nature Preserve	2		**ABC**	E				D		D
Radio Telescope	3			**ACD**		**BE**				
Redwood City Marina	4		CD		**ABDE**		E			
Bridge Toll Plaza	5						**ABD**		**DCE**	
Drive-In Theatre	6			B		A	C			E
Arts and Crafts Garden Plaza	7							**ABCE**		
Church	8				C				AB	
Rinconada Park	9		CE							**AB**

Of the 45 selections (5 judges, 9 choices), 24 were correct. Bold type indicates the description chosen most often for each place visited. Correct choices lie on the main diagonal. The number of correct matches by Judges A through E is 7, 6, 5, 3, and 3, respectively. The expected number of correct matches from the five judges was five; in the experiment 24 such matches were obtained. The *a priori* probability of such an occurrence by chance, conservatively assuming assignment without replacement on the part of the judges, is $P = 8.10^{-10}$.

not otherwise associated with the research. The judges were asked to match the nine locations, which they independently visited, against the typed manuscripts of the tape-recorded narratives of the remote viewer. The transcripts were unlabelled and presented in random order. The judges were asked to find a narrative which they would consider the best match for each of the places they visited. A given narrative could be assigned to more than one target location. A correct match requires that the transcript of a given date be associated with the target of that date. Table 2 shows the distribution of the judges' choices.

Among all possible analyses, the most conservative is a permutation analysis of the plurality vote of the judges' selections assuming assignment without replacement, an approach independent of the number of judges. By plurality vote, six of the nine descriptions and locations were correctly matched. Under the null hypothesis (no remote viewing and a random selection of descriptions without replacement), this outcome has an *a priori* probability of $P = 5.6 \times 10^{-4}$, since, among all possible permutations of the integers one through nine, the probability of six or more being in their natural position in the list has that value. Therefore, although Price's descriptions contain inaccuracies, the descriptions are sufficiently accurate to permit the judges to differentiate among the various targets to the degree indicated.

EEG EXPERIMENTS

An experiment was undertaken to determine whether a physiological measure such as EEG activity could be used as an indicator of information transmission between an isolated subject and a remote stimulus. We hypothesised that perception could be indicated by such a measure even in the absence of verbal or other overt indicators.[6,7].

It was assumed that the application of remote stimuli would result in responses similar to those obtained under conditions of direct stimulation. For example, when normal subjects are stimulated with a flashing light, their EEG typically shows a decrease in the amplitude of the resting rhythm and a driving of the brain waves at the frequency of the flashes[8]. We hypothesised that if we stimulated one subject in this manner (a sender), the EEG of another subject in a remote room with no flash present (a receiver), might show changes in alpha (9–11 Hz) activity, and possibly EEG driving similar to that of the sender.

We informed our subject that at certain times a light was to be flashed in a sender's eyes in a distant room, and if the subject perceived that event, consciously or unconsciously, it might be evident from changes in his EEG output. The receiver was seated in the visually opaque, acoustically and electrically shielded double-walled steel room previously described. The sender was seated in a room about 7 m from the receiver.

To find subjects who were responsive to such a remote stimulus, we initially worked with four female and two male volunteer subjects, all of whom believed that success in the experimental situation might be possible. These were designated 'receivers'. The senders were either other subjects or the experimenters. We decided beforehand to run one or two sessions of 36 trials each with each subject in this selection procedure, and to do a more extensive study with any subject whose results were positive.

A Grass PS-2 photostimulator placed about 1 m in front of the sender was used to present flash trains of 10 s duration. The receiver's EEG activity from the occipital region (O_Z), referenced to linked mastoids, was amplified with a Grass 5P-1 preamplifier and associated driver amplifier with a bandpass of 1–120 Hz. The EEG data were recorded on magnetic tape with an Ampex SP 300 recorder.

On each trial, a tone burst of fixed frequency was presented to both sender and receiver and was followed in one second by either a 10 s train of flashes or a null flash interval presented to the sender. Thirty-six such trials were given in an experimental session, consisting of 12 null trials—no flashes following the tone—12 trials of flashes at 6 f.p.s. and 12 trials of flashes at 16 f.p.s., all randomly intermixed, determined by entries from a table of random numbers. Each of the trials generated an 11-s EEG epoch. The last 4 s of the epoch was selected for analysis to minimise the desynchronising action of the warning cue. This 4-s segment was subjected to Fourier analysis on a LINC 8 computer.

Spectrum analyses gave no evidence of EEG driving in any receiver, although in control runs the receivers did exhibit driving when physically stimulated with the flashes. But of the six subjects studied initially, one subject (H. H.) showed a consistent alpha blocking effect. We therefore undertook further study with this subject.

Data from seven sets of 36 trials each were collected from this subject on three separate days. This comprises all the data collected to date with this subject under the test conditions described above. The alpha band was identified from average spectra, then scores of average power and peak power were obtained from individual trials and subjected to statistical analysis.

Of our six subjects, H. H. had by far the most monochromatic EEG spectrum. Figure 3 shows an overlay of the three averaged spectra from one of this subject's 36-trial runs, displaying changes in her alpha activity for the three stimulus conditions.

Mean values for the average power and peak power for each

Table 3 EEG data for H.H. showing average power and peak power in the 9–11 Hz band, as a function of flash frequency and sender

Flash Frequency	0	6	16	0	6	16
Sender	Average Power			Peak Power		
J.L.	94.8	84.1	76.8	357.7	329.2	289.6
R.T.	41.3	45.5	37.0	160.7	161.0	125.0
No sender (subject informed)	25.1	35.7	28.2	87.5	95.7	81.7
J.L.	54.2	55.3	44.8	191.4	170.5	149.3
J.L.	56.8	50.9	32.8	240.6	178.0	104.6
R.T.	39.8	24.9	30.3	145.2	74.2	122.1
No sender (subject not informed)	86.0	53.0	52.1	318.1	180.6	202.3
Averages	56.8	49.9	43.1	214.5	169.8	153.5
	−12%	−24% ($P < 0.04$)		−21%	−28% ($P < 0.03$)	

Each entry is an average over 12 trials

of the seven experimental sets are given in Table 3. The power measures were less in the 16 f.p.s. case than in the 0 f.p.s. in all seven peak power measures and in six out of seven average power measures. Note also the reduced effect in the case in which the subject was informed that no sender was present (Run 3). It seems that overall alpha production was reduced for this run in conjunction with the subject's expressed apprehension about conducting the experiment without a sender. This is in contrast to the case (Run 7) in which the subject was not informed.

Siegel's two-tailed t approximation to the nonparametric randomisation test[9] was applied to the data from all sets, which included two sessions in which the sender was removed. Average power on trials associated with the occurrence of 16 f.p.s. was significantly less than when there were no flashes ($t = 2.09$, d.f. $= 118$, $P < 0.04$). The second measure, peak power, was also significantly less in the 16 f.p.s. conditions than in the null condition ($t = 2.16$, d.f. $= 118$, $P < 0.03$). The average response in the 6 f.p.s. condition was in the same direction as that associated with 16 f.p.s., but the effect was not statistically significant.

Spectrum analyses of control recordings made from saline with a 12 kΩ resistance in place of the subject with and without the addition of a 10 Hz, 50 μV test signal applied to the saline solution, revealed no indications of flash frequencies, nor perturbations of the 10 Hz signal. These controls suggest that the results were not due to system artefacts. Further tests also gave no evidence of radio frequency energy associated with the stimulus.

Subjects were asked to indicate their conscious assessment for each trial as to which stimulus was generated. They made their guesses known to the experimenter via one-way telegraphic communication. An analysis of these guesses has shown them to be at chance, indicating the absence of any supraliminal cueing, so arousal as evidenced by significant alpha blocking occurred only at the noncognitive level of awareness.

We hypothesise that the protocol described here may prove to be useful as a screening procedure for latent remote perceptual ability in the general population.

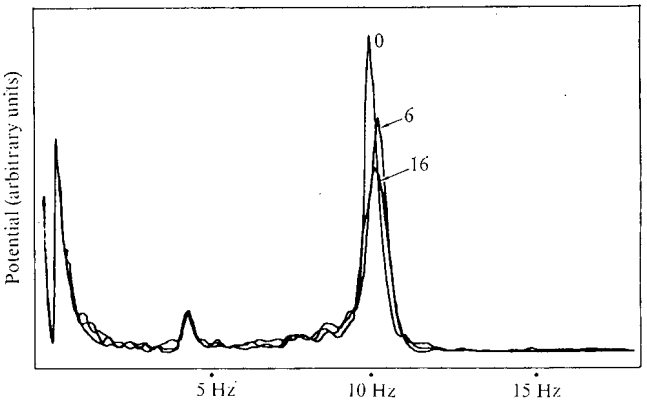

Fig. 3 Occipital EEG spectra, 0–20 Hz, for one subject (H. H.) acting as receiver, showing amplitude changes in the 9–11 Hz band as a function of strobe frequency. Three cases: 0, 6, and 16 f.p.s. (12 trial averages).

CONCLUSION

From these experiments we conclude that:

- A channel exists whereby information about a remote location can be obtained by means of an as yet unidentified perceptual modality.
- As with all biological systems, the information channel appears to be imperfect, containing noise along with the signal.
- While a quantitative signal-to-noise ratio in the information-theoretical sense cannot as yet be determined, the results of our experiments indicate that the functioning is at the level of useful information transfer.

It may be that remote perceptual ability is widely distributed in the general population, but because the perception is generally below an individual's level of awareness, it is repressed or not noticed. For example, two of our subjects (H. H. and P. P.) had not considered themselves to have unusual perceptual ability before their participation in these experiments.

Our observation of the phenomena leads us to conclude that experiments in the area of so-called paranormal phenomena can be scientifically conducted, and it is our hope that other laboratories will initiate additional research to attempt to replicate these findings.

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The stability of a feasible random ecosystem

THE weight of the evidence, and the beliefs of most biologists, seem to support the view[1] that ecosystems tend to be more stable, the larger the number of interacting species they contain. It is puzzling, therefore, that a variety of mathematical models of complex ecosystems appear to give the contrary answer: that complexity makes for instability[2].

Prominent among such models is the complex system with random interactions, studied in various forms by Gardner and Ashby[3] and May[4]; but their results cannot be applied as they stand to ecological systems. In an ecosystem, the interacting variables are species populations (or species biomass) which cannot take on negative values. Thus, for example, the equilibrium population values must be positive, and it is convenient to denote this necessary property of an ecosystem model by saying that it must be 'feasible'.

The work referred to imposed no such constraint on equilibrium populations in the samples considered. It is of some interest, therefore, to examine the stability of a random model capable of representing ecosystems, by imposing the restriction that the sample be feasible.

I report here the results of computer calculations on such a model. The interaction equations were of the well-known quasi-linear type, in which the rate of fractional increase of a species population is a linear function of the current populations in all T species. That is, the number N_i in the ith species obeys

$$dN_i/dt = N_i (b_i + \Sigma_j a_{ij} N_j).$$

All birth rates b_i were taken as 1, and the self-regulating coefficients a_{ii} as -1. The feasibility requirement was that the

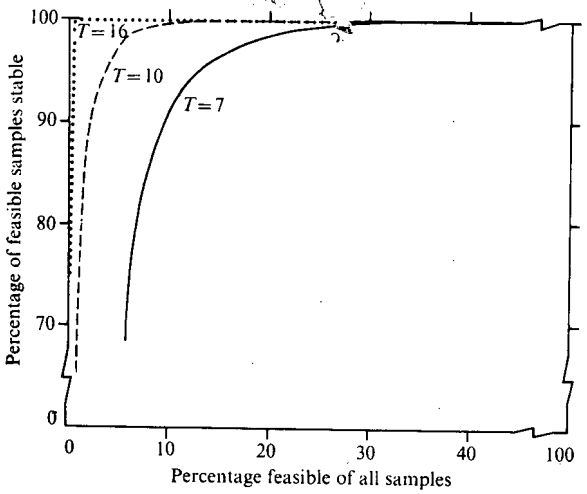

Fig. 1 Stability of feasible samples.

solutions for the equilibrium populations N^*_i, obtained by equating the dN_i/dt to zero, should be strictly positive. The stability matrix S is given by $S_{ij} = N^*_i a_{ij}$; S is unstable if it has an eigenvalue with positive real part. The interaction coefficients were chosen to have a fixed magnitude z but random sign (+ or − equally likely), with all T species uniformly self regulated. A minimum of 2,000 samples were examined for each value of T and z.

When all samples were accepted—whether feasible or not—the results for $T = 7$, 10 and 16 agreed qualitatively with the related (though not identical) models of refs 3 and 4. That is, for z small, essentially all samples were stable, but the stable fraction tended to zero as z approached T^{-1}.

On examining the stability of the feasible samples only, however, a significantly different behaviour emerges. We see from Fig. 1 that feasible samples are generally stable, the stable percentage departing from close to 100% only when the occurrence of a feasible sample is in any case rare (that is, when z is large). Moreover, this tendency to stability increases with the number T of interacting species. (It should be noted that the curves in Fig. 1 contain no extrapolated portions, but are constructed by interpolating between computed points of negligible Monte Carlo error.)

The results displayed suggest the conclusion that, for this model type, almost all feasible systems are stable when the number of interacting species increases without limit. (The curve for $T = 16$ is already barely distinguishable from such a step function.)

The results confirm previous findings (both numerical[3] and analytical[4]), in showing that both feasibility and stability are rare when $z > T^{-1}$. It is not unreal, however, to expect average interaction strength to decrease as the number of interactions increases.

It is easy to see why the general conclusions emerging differ from those of refs 3 and 4, when these are specialised to ecosystems. The latter studies worked directly on the stability matrix, and thus could not impose any feasibility requirement (this must be derived from the solutions of the original interaction equations, which do not appear in their approach). Now, unfeasible samples contain at least one species with a negative equilibrium population; this simply means, biologically, that after a time the species becomes extinct. But, if this extinction is ignored, such 'ghost species' are being implicitly retained in the stability examination and exert a profoundly destabilising effect. In the quasi-linear model above, for example, they 'live on' as an unregulated, exponentially increasing component of the system. The diagonal element of the stability matrix is $-N^*_i$, which is positive when $N^*_i < 0$. Thus the ghost species figures in it like a real species (that is, one with a positive number at equilibrium) with $a_{ii} = +1$, that is, with an eventual population increase proportional to N_i[2].

The results cited above show that, when these 'mathematical ghosts' are excluded, the model shows an actual increase of stability in the larger systems.

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Objective evaluation of auditory evoked EEG responses

THE averaging of often very small responses is widely used in physiology, psychology and clinical medicine to evaluate stimulus threshold levels. It is sometimes difficult to recognise responses in the average waveform because of residual activity, so a means of improving sensitivity and objectively recognising positive responses would be useful. We have previously found[1] that the averaged responses (ERA) evoked in the electroencephalogram (EEG) of a subject by auditory stimuli were produced by imposing a phase constraint on the different Fourier harmonics of the spontaneous EEG, without necessarily influencing their amplitudes.

Merely imposing the phase-spectrum characteristic of a response to high-level stimuli on length of unstimulated spontaneous EEG from the same or another individual, creates a waveform similar to and highly correlated with a true average of responses to high-level stimuli and a similar result is produced by imposing the appropriate phase spectrum on lengths of pseudo-random white Gaussian noise instead of on spontaneous EEG. These observations provide a convenient basis for the objective detection of the existence of positive responses to given stimuli and for the determination of auditory threshold levels.

Our previous findings concerned the average, rather than the individual, evoked response but clearly must reflect phenomena in the separate lengths of EEG following individual stimuli. If so, it should be possible to demonstrate a phase-constraint operative on the various harmonic components of the individual post-stimulus lengths of EEG and such a demonstration would be useful in suggesting the basis of an objective assessment of the occurrence of response. Unfortunately, phase spectra do present difficulty of interpretation and in the present context, may be liable to some error if unnecessarily long record lengths are used. One problem is that phase values are restricted to the range −180° to +180°, and as a result of the repeated recurrence of the principal range of angles that can occur, averaging phase-spectral values for any given harmonic component, in successive sweeps, is usually unsatisfactory. We have therefore investigated instead whether the ensemble distribution of phase values for each harmonic component shows any aggregation, or if it shows the uniform distribution characteristic of uncontrolled phases as seen in unstimulated records.

Phase values have been calculated for each of the first ten Fourier harmonic components and classified into four equal-size groups covering the full 360° range of possible values. The null hypothesis leads to a uniform distribution of phase values so that for 60 sweeps, the expected number of values is 15 in each class. A χ^2 test with d.f.=3 can then be applied to test the null hypothesis (that is, no

phase control), for each harmonic separately. The results show that for high-level stimuli, the null hypothesis must be rejected on the basis of one or more harmonics ($P<0.01$), but for stimuli that are evidently subthreshold according to both subjective judgment and appearance of the averaged response waveform, the null hypothesis holds. Intermediate levels lead to χ^2 values that broadly decrease in significance as stimulus level decreases and the 5% level occurs near the subjective threshold. For high-level stimuli which produce an evident response, most of the harmonics exhibit the phase constraint, but as the stimulus level is reduced towards threshold, significant aggregation of phase values is confined only to a few harmonics. The effects reported here have been studied for increasing lengths of post stimulus record, starting with about 0.32 s and ultimately reaching the full length of our records (0.94 s post stimulus). The existence of a phase constraint is increasing evident with increasing record length; the effect often fails altogether, however, when the record length is allowed to exceed about 0.8 s. This observation suggests that after about 0.75 s any phase control of spontaneous EEG activity diminishes, allowing fully spontaneous conditions to be generally restored beyond this point. If the record used for analysis did include, for this reason, a segment of spontaneous activity, the appearance of the phase constraint would then be lost.

These findings apply in each of the trials with different tonal or click stimuli for all the six subjects (three adults, three children) reported previously[1], and similarly to each of the records from three further subjects that we have so far examined. Thus, a non-subjective and quantitative system of auditory assessment would seem to be feasible, utilising phase-spectral measurements alone. This approach, however, implicitly utilises some information about the amplitude spectrum of the signal, in that low amplitude Fourier components are neglected (by restricting analysis to the first ten or fewer harmonics), and we would not rule out the possibility of utilising further amplitude spectral information to supplement the indications provided by a phase analysis.

We would also remark that the characterisation of these signals in phase-spectral terms is an abstraction to describe a mechanism that is perhaps more realistically envisaged as a synchronisation process in the time domain, rather than as a phase-constraint in the frequency domain.

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Imprinting and exploration of slight novelty in chicks

Shortly after hatching, domestic chicks will respond socially to a wide range of moving or conspicuous objects. As a result of 'imprinting', their social behaviour is increasingly limited to objects with which they have had visual experience. In general, the longer that chicks have been exposed to an object, the more strongly they prefer it when given a choice between it and something else[1–5]. Some evidence, however, suggests that in the early stages of imprinting, the trend towards a highly restricted preference is temporarily reversed and some birds may even prefer the unfamiliar object[6,7].

Here we report that day-old chicks will even work to expose themselves to novel stimulation at an early stage in imprinting. At that stage, by changing the characteristics of a visual stimulus when a chick stepped onto a pedal, it was possible to increase the likelihood that it would step on to the pedal again.

Bateson and Reese[8] found that day-old chicks hatched and reared in the dark would rapidly learn to press a pedal which turned on a flashing rotating light. We have used a similar technique in this study but with two important differences: first, the chicks had been exposed to a flashing, rotating light for varying amounts of time before the experiment; second, the familiar stimulus was turned on when a bird was not on the pedal. As soon as the chick stepped on the pedal the colour of the stimulus was changed. In other words, the bird was exposed to a novel stimulus for as long as it stood on the pedal.

The apparatus consisted of a chamber measuring 37.5 by 34.5 by 33 cm. Two of the walls were made of hardboard and the front and back walls of 1.27-cm wire mesh. Two pedals were set into the floor; each measured 4.8 cm square and was placed 2.0 cm from the wire mesh front and 2.6 cm from one of the sides. All the apparatus was painted matt black except for the pedals which were matt white. The temperature 5 cm above the floor of the apparatus was 23.5–26.5° C. The stimulus was placed 60 cm in front of the wire mesh and midway between the two pedals. It

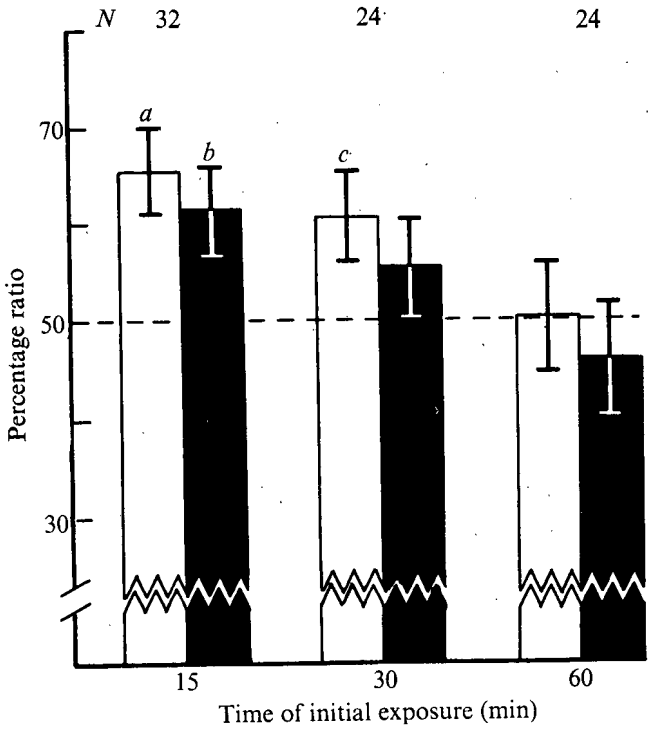

Fig. 1 Means and s.e. for the total presses by day-old chicks of a pedal altering a flashing light from a familiar to unfamiliar colour. The pedal presses are expressed as percentages of the total presses of that pedal and another symetrically placed pedal having no effect on the light. Open bars are percentage durations of pressing and solid bars are percentage frequencies of pressing. The chicks were previously exposed to the familiar light for 15, 30 or 60 min. Departures from the chance level of 50% are indicated as follows: *a*, $t = 3.29$, $P < 0.01$; *b*, $t = 2.56$, $P < 0.02$; *c*, $t = 2.14$, $P < 0.05$.

consisted of a translucent cylinder 15.3 cm in diameter and 16 cm in length rotating at 45 r.p.m. on a horizontal axis. The cylinder was covered with a chequerboard pattern (2-cm squares). A primary red and primary yellow filter were fitted inside the cylinder, each over a 45 W bulb. The bulbs could be individually lit to make the whole cylinder appear as either red or yellow checks; the background was matt black. When one pedal was pressed the colour of the cylinder changed; the other pedal had no effect on the stimulus and was used to measure any general changes in activity.

Eighty Ross 'Chunky' chicks were hatched in the laboratory and individually exposed to constant white light for 50 min. When they were 14–22 h old, half were individually exposed in activity wheels[9] to a flashing rotating light like the red stimulus in the operant conditioning situation and half were exposed to one like the yellow stimulus. The chicks were exposed for 15, 30 or 60 min and 3 min later were separately placed in the operant conditioning box. The experimental design was balanced so that for half the chicks the pedal changing the colour of the stimulus was on the right hand side and for the other half it was on the left hand side. Each chick was left in the apparatus for 50 min.

The results are shown in Fig. 1. The presses of the pedal changing the colour of the light are expressed as a percentage of the total presses of both pedals; if they were responding at the chance level the ratio would have been 50%. The absolute measures of pedal pressing were the same for all groups. In Fig. 1 percentages are given for both the number of presses in the 50-min tests and the total time spent pressing the pedals. It can be seen that after 15 min previous experience with the familiar stimulus the chicks pressed the pedal which changed the colour of the light more frequently and for longer than they did the other pedal. The red-trained birds did not differ from the yellow-trained birds in the extent to which they worked for change. In the red-trained chicks exposed for 30 min, the effect was much less clearly marked and by 60 min the birds in both groups were responding at the chance level. The overall decline in the ratios is statistically significant for both measures (for response number Pearson $r=-0.242$, $P<0.05$, for response duration, $r=-0.240$, $P<0.05$).

Differential pressing of the two pedals cannot be attributed to an initial preference for one stimulus over the other regardless of previous experience because the experimental design was balanced and the subgroups did not differ. Similarly their behaviour cannot be explained in terms of a preference for the position of one pedal over the other. We believe that the results unequivocally support earlier suggestions that chicks prefer novel objects at an early stage in imprinting[6]. Moreover, they show that the birds will actively work to expose themselves to slightly novel stimulation. The biological advantages of such behaviour may lie in the very complexity of what the young bird has to learn under natural conditions. The ability to recognise a parent from all likely angles almost certainly requires more information than could possibly be acquired as the result of learning the characteristics of a single view of that parent. The transformation from front view to side view and from side view to back view could not easily be accomplished by mechanisms ordinarily understood as being involved in perceptual constancy[7]. Therefore, if a young bird must recognise its parent from a variety of angles it must have first seen its parent from all those angles. This would in part be accomplished by rotations of the parent as it went about its business; but the process of attachment would be accelerated if, in addition, the young moved round the parent after familiarising themselves with one view. Such a process of working for slight novelty balanced against a growing restriction of preference for what is familiar would not only make good biological sense, it would account for the birds preferring the unfamiliar object only in the early stages of imprinting[10].

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Microbial activation of prophenoloxidase from immune insect larvae

Interest in the infection of insects by parasites has been generated by the pathogenic potential of the parasite to the insect itself, or to a vertebrate host to which the parasite may be transmitted. For example, because of the effects of malaria on man, the relationships between *Plasmodia* and various mosquito species have received much attention[1,2]. An important part of the host-parasite relationship is the defence system of the host. In insects, phenoloxidase (*o*-diphenol: O_2 oxidoreductase, EC 1.10.3.1.) has been suggested to be at least partially responsible for insect immunity and parasite resistance[3–5].

The enzyme produces quinones which can react with proteins[6] and with the thiol and amino groups of other compounds[6–8]. Quinones can be bactericidal[9]. When quinones are present in excess, the black pigment melanin is formed[6]. A layer of melanin is frequently observed deposited on parasites by insects as part of their resistance. The exact reactions of quinones with parasite tissues have not been determined, but by blocking quinone formation with phenylthiourea an insect's parasite resistance can be suppressed[5]. Melanin deposition is often associated with insects' cellular response[5], but can also be a strictly humoral response[10]. In some cases of unsuccessful malaria transmission by mosquitoes there is an apparent melanisation manifest in Ross black spores and degenerate oocysts[1,2]. For quinones to be involved in resistance to an insect parasite, however, control of phenoloxidase activity would be essential. Quinones randomly reacting with the insect host would be ineffective as antiparasitic agents. Furthermore, large amounts of active phenoloxidase can kill insects[11]. As a contribution to this problem, I describe here the activation of prophenoloxidase in the plasma of immune[12,13] *Galleria mellonella* larvae by microbial products.

For immunisation, larvae (each larger than 200 mg) were injected with 1.0-μg doses of *Shigella flexneri* lipopolysaccharide B (Difco)[13] and incubated at 23° C. Control larvae were uninjected. Haemolymph was collected 18–24 h after immunisation and pooled in a CO_2 environment; at

Table 1 Enzyme activity in $(NH_4)_2SO_4$ fractions of immune plasma

$(NH_4)_4SO_4$ fraction*	Protein	Phenoloxidase‡				Lysozyme
		Zymosan activation		α-Chymotrypsin activation		
	mg g^{-1} lyophilisate†	U g^{-1} lyophilisate	Specific activity§	U g^{-1} lyophilisate	Specific activity§	U g^{-1} lyophilisate‖
0–40% ppt., fraction *A*	10	0	0	43,950	4,395	0
40–50% ppt.	85	0	0	21,850	257	0
50–60% ppt.	140	0	0	50	0	300
60% Supernantant, fraction *B*	66	0	0	0	0	14,300
Whole plasma¶	558	144,045	258	153,745	276	—

* Plasma was prepared from reconstituted haemolymph as described in the text. Fractions were prepared by the addition of solid salt[18] (100% saturation = 70.6 g per 100 ml). Precipitates were dissolved in 0.01 M KPO_4^{-2}, *p*H 6.0 (the *p*H and ionic concentration—as determined by freezing point depression—of the plasma). Before testing enzymatic activities all fractions were dialysed against this buffer to remove the salt.

† Assayed by the method of Lowry *et al.*[19].

‡ 0.20 ml of a fraction was mixed with 0.10 ml of activator in water: α chymotrypsin, 6 mg ml^{-1}: zymosan, 1 mg ml^{-1}. Mixtures were incubated for 5 min at 30° C, then assayed for phenoloxidase. Values for water controls have been subtracted.

§ U mg^{-1} protein.

‖ ΔA_{450} = −0.001 per min at 30° C of a suspension of *Micrococcus lysodeikticus* was one lysozyme unit[20].

¶ No salt added, no dialysis.

least 20 larvae were used for each pool. Care was taken to avoid gross contamination of haemolymph with fat body tissue. To quantitate properly phenoloxidase activity per amount of haemolymph and to facilitate handling, pooled haemolymph was quick frozen with acetone-dry ice, lyophilised and stored at −20° C. Haemolymph from control larvae was collected and stored in an identical manner.

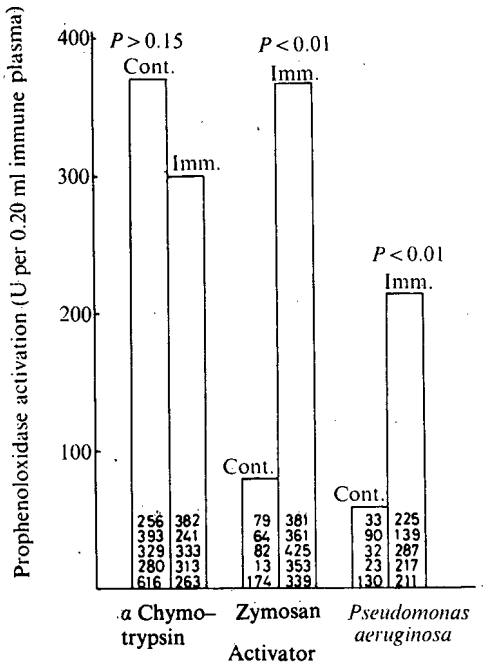

Fig. 1 Activation of prophenoloxidase in control (Cont) and immune (Imm) plasmas by various materials. The values indicated by the bars are the average amounts of prophenoloxidase found in five different determinations. A determination used 0.20 ml of plasma from a different pool of control or immune haemolymph mixed with 0.01 ml of activator solution or suspension, incubated for 5 min at 30° C, then assayed for phenoloxidase. (See text for assay technique.) Results for individual determinations are listed within each bar. With no activator materials present—water controls—the phenoloxidase activities of plasmas ranged from zero to two units; these values have been subtracted. Statistical comparisons are between control and immune plasmas. α-Chymotrypsin (Nutritional Biochemicals Corp., three times crystalline) 6 mg ml^{-1} water. Zymosan[16] (General Biochemicals) 1 mg ml^{-1} water. *Pseudomonas aeruginosa* P11–1[12] was grown in nutrient broth +1% glucose and collected at 24 h by centrifugation, 8,000*g* for 15 min. Cells were washed with 0.85% NaCl, then brought to A_{550} = 1.1, a concentration of about 5×10^9 bacteria per ml and frozen. The cell suspension was thawed, resuspended in water and refrozen. It was rethawed and heated at 100° C for 30 min. After cooling, 0.10 ml aliquots were used to activate prophenoloxidase. None of these activator materials had inherent phenoloxidase activity.

My preliminary experiments and those of Evans[14] have shown that haemolymph phenoloxidase occurs as the proenzyme in plasma and can be activated by material from haemocytes. To test for other types of activation, lyophilised haemolymph was reconstituted with distilled water to a concentration of 10 mg solids per ml. This was 1/17 to 1/18 the usual concentration of solids in fresh haemolymph, and its purpose was to slow spontaneous activation of prophenoloxidase by haemocytes, so that plasma could be collected before activation. The plasma and cellular fractions were separated by centrifuging whole reconstituted haemolymphs at 650*g* for 15 min at 4° C. The supernatant fluids—the plasmas—were removed and used immediately. When water was added to the haemocytes in the lyophilised material no lysis was observed microscopically. Also, haemocyte concentrations were calculated, with correction factors for dilutions, to be 18,590 mm^{-3} for immune and 40,040 mm^{-3} for control reconstituted haemolymph, slightly higher than those reported by Stephens[15] for immune and control fresh haemolymph. These observations tend to preclude massive haemolysis during handling although the release of some haemocyte materials into the plasmas was possible.

Phenoloxidase activity was measured by spectrophotometric determinations of initial reaction rates with a Gilford recording spectrophotometer. For a standard assay, 0.30 ml of test material was mixed with 4.7 ml of 0.1 M KPO_4^{2-}, *p*H 6.4, containing 20 μmol of 4-methylcatechol and 40 μmol of 4-hydroxyproline ethyl ester. 4-Methylcatechol was enzymatically oxidised to its quinone. The quinone reacted nonenzymatically with 4-hydroxyproline ethyl ester to form a stable product, 4-(4′-hydroxy-2′-carbethoxy-1′-pyrrolidyl)-5-methyl-*o*-benzoquinone, with a λ_{max} at 520 nm (ref. 7). In the system used, with excess substrates, ΔA_{520} was directly proportional to enzymatic activity. One phenoloxidase unit was defined as ΔA_{520} = +0.001 per min at 30° C; the maximum rate observed during the first 2 min after the initiation of the reaction was used. By measuring the phenoloxidase activity of plasmas incubated with and without activator materials, prophenoloxidase activation was quantitated.

Immune plasmas showed significantly more prophenoloxidase activation than control plasmas when both were incubated with either zymosan (a yeast polysaccharide)[16] or a preparation of damaged *Pseudomonas aeruginosa* (Fig. 1). After treatment with α chymotrypsin (EC 3.4.4.5.) both control and immune plasmas showed a high degree of

prophenoloxidase activation, similar to that in immune plasmas treated with the microbial products.

The activator(s) associated with zymosan and *P. aeruginosa* did not have esterase activity towards benzoyl-L-tyrosine ethyl ester when tested at *p*H 6.0 (the *p*H of the plasmas) or at *p*H 7.8. The activator(s) in zymosan suspensions was associated not only with particles, but also with the particle-free solution. It (they) passed through a 0.22-μm filter, but not through a dialysis membrane. The zymosan activator(s) was stable at 100° C for 3 h, but after 3 h in 0.2 M H_2SO_4 at 100° C and then neutralisation with $Ba(OH)_2$ it was not found when assayed. Activation associated with *P. aeruginosa* was not manifest unless the cells were damaged by physical means or by pretreatment with egg white lysozyme (EC 3.2.1.17.) (Fig. 2). Egg white lysozyme does not lyse or kill *P. aeruginosa*, but alters its cell wall[17].

Immune plasma was fractionated with $(NH_4)_2SO_4$ (Table 1). The material precipitated with 40% saturation (fraction *A*) contained most of the prophenoloxidase. The material still in solution after 60% saturation (fraction *B*) had most of the lysozyme activity. Prophenoloxidase in fraction *A* was activated by α chymotrypsin, but not by zymosan alone; however, the prophenoloxidase in fraction *A* could be activated by zymosan if fraction *B* was also added. If egg white lysozyme was substituted for fraction *B*, activation did not occur. The K_ms of phenoloxidase activated by zymosan and α chymotrypsin for 4-methylcatechol were similar, 1.20 and 1.07 mM respectively. Figure 3 shows that when prophenoloxidase was diluted before activation by zymosan there was an exponential relationship between proenzyme concentration and activity, while dilution of the enzyme after activation gave an almost linear relationship.

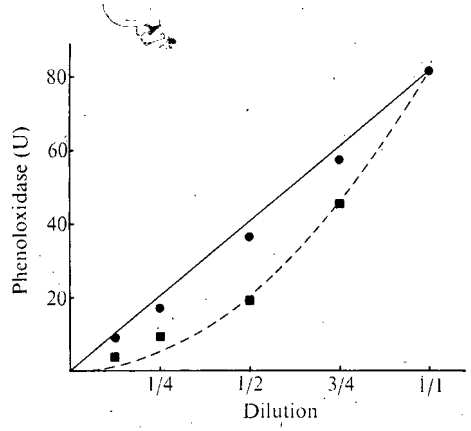

Fig. 3 Effect of prophenoloxidase concentration in the zymosan activation mixture on the resulting phenoloxidase activity. ■, Prophenoloxidase diluted before activation ($y=81x^2$): fraction *A* was brought to various dilutions with 0.01 M KPO_4^{2-}, *p*H 6.0. 0.10 ml of a dilution was mixed with 0.10 ml of fraction *B*, and 0.10 ml of zymosan suspension, 1 mg ml^{-1} water. Each of these mixtures was incubated for 5 min at 30° C, then assayed for phenoloxidase. ●, Phenoloxidase diluted after activation ($y=81x$): fraction *A*, fraction *B*, and zymosan suspension were mixed 1:1:1 (v:v:v), then incubated at 30° C. After 5 min, various dilutions of this mixture were made using a mixture of 0.01 M KPO_4^{2-}, *p*H 6.0, and zymosan suspension, 2:1 (v:v). Immediately after all dilutions had been prepared, 0.30 ml aliquots were assayed for phenoloxidase activity. These assays were completed within 15 min.

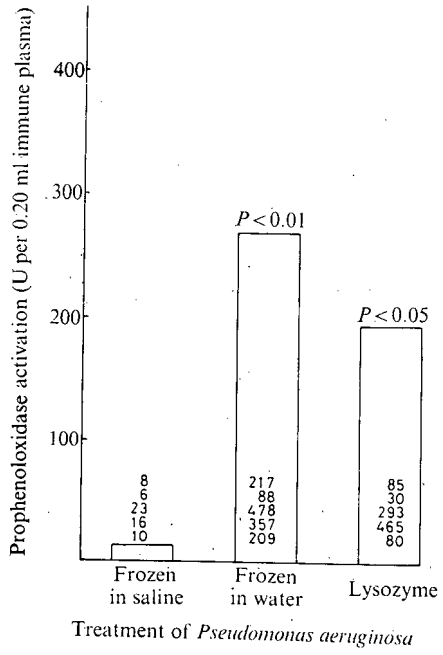

Fig. 2 Manifestation of prophenoloxidase activator(s) in *Pseudomonas aeruginosa* after various treatments. The activator(s) was determined by the activation of prophenoloxidase in immune plasmas. The assay procedures were the same as for Fig. 1. *P. aeruginosa* cells frozen in 0.85% NaCl saline (see Fig. 1), resuspended in water, but not refrozen or heated, served as controls. Statistical comparisons are between these cells and those submitted to other treatments. The cell suspension frozen in water was the *P. aeruginosa* preparation used for Fig. 1. Cells treated with lysozyme were frozen in saline, resuspended in 0.1 M KPO_4^{2-}, *p*H 7.0, with 10 mg lysozyme (Sigma, from egg white, three times crystallised) ml^{-1}, incubated 20 min at 30° C, and then resuspended in water. Bacterial concentrations were the same for all three suspensions.

Activation of prophenoloxidases is associated with the aggregation of enzyme subunits[18,21]. An aggregation mechanism is consistent with the concentration effect shown in Fig. 3. Haemolymph lysozyme in fraction *B* may partially digest zymosan material which then acts as a site for the adsorption, aggregation and activation of prophenoloxidase subunits. This would explain the differences observed between control and immune plasmas in Fig. 1, since immune haemolymph contains larger concentrations of lysozyme[22]. Another hypothesis is that zymosan activates a proteolytic enzyme in fraction *B*, as it does in complement[23]. The proteolytic enzyme would in turn activate the prophenoloxidase.

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Elevation of total serum IgE in rats following helminth parasite infection

HELMINTH parasites provide the most potent stimulus known for the biosynthesis of IgE. In every species which has been studied, infections with parasitic worms lead to the production of high levels of reaginic or IgE antibodies against parasite antigens[1,2] and, in man, greatly elevated levels of total IgE occur in most individuals infected with any one of a variety of helminths including *Ascaris lumbricoides*[3], *Capillaria philipinensis*[4] and ancylostomes[5].

Experiments in the rat have indicated how the IgE stimulating effect of helminth parasites may operate. In this species, infections with *Nippostrongylus brasiliensis* or *Fasciola hepatica*, as well as stimulating parasite specific IgE antibodies, cause nonspecific potentiation of unrelated IgE responses to such antigens as egg albumen, keyhole limpet haemocyanin, and house dust[6–8]. Present evidence indicates that live worms produce a factor which, acting via T cells[9], has the capacity to stimulate those B cells which have been programmed for IgE production by previous immunisation. In so far as IgE responses against different antigens may be simultaneously potentiated[8] it seems likely that all the existing IgE responses of the animal are affected.

Here we now present evidence which supports this. We have compared the level of total serum IgE in rats following conventional immunisation with that of rats whose response has been potentiated by subsequent infection with helminth parasites. In the latter, the amount of total serum IgE is much greater, and far more than can be ascribed to IgE antibodies specific for the immunising antigen or the parasite.

Until recently it has not been possible to estimate total serum IgE in the rat, or indeed in any other species but man, for lack of specific antiserum to this immunoglobulin. The recent discovery of a strain of rats bearing IgE immunocytomas[10,11] has overcome this difficulty since they provide a source of myeloma protein from which purified IgE can be isolated in relatively large amounts. Such a purified IgE preparation has been used to induce specific antiserum to rat IgE and also as a reference preparation in an assay for rat total serum IgE using the radioactive single-radial-diffusion technique of Rowe[12].

The specific immunological reagents were prepared as follows: The purification of rat IgE from the serum or rats bearing IgE secreting immunocytomas was as previously described[10] with the following modifications. Ammonium sulphate precipitation was performed at 50% final concentration, and as a supplementary step preparative agarose electrophoresis was done in 1% agarose equilibriated with 63 mM sodium barbiturate, 15 mM Barbital, 1.8 mM calcium lactate buffer, adjusted to *p*H 8.6 with HCl. The electrophoresis was carried out for 1 h at 20 V cm^{-1}. After electrophoresis, the position of the monoclonal IgE was located by comparison with a fixed and stained agarose gel electrophoretic pattern of the same IgE preparation. The agarose-containing myeloma IgE was cut out and removed from the slide and the proteins were extracted from the gel by pressure. Goat antiserum to rat IgE was obtained by immunisation with purified antigen (IR2 monoclonal protein) emulsified in complete Freunds adjuvant. Antisera were absorbed with a solid immunoabsorbant[13] made of serum from germ-free rats. Antiserum to goat IgG was prepared by immunisation of rabbits with goat IgG (prepared by the caprylic acid method)[14] in complete Freunds adjuvant. The antiserum was also purified by the caprylic acid technique and the resultant IgG fraction was labelled with ^{125}I by the method of McConahey and Dixon[15].

Dilutions of anti-rat IgE in the agar from 1:100 to 1:4,000 could be used. The last dilution gave the greatest sensitivity enabling estimation in the range of 0.35-22.24 μg IgE ml^{-1}. The reference preparation (IR162 Temoin) contained 5.7 mg ml^{-1} of rat IgE.

Outbred female Hooded Lister rats weighing 150–200 g were used in the experiments described here. Twenty normal rats (Group 1) were bled before treatment and were then

Table 1 Reaginic antibody titre and total serum IgE (mean ± s.e.) in serum from normal, immunised infected and potentiated rats

Group	Treatment of rats	Passive cutaneous anaphylaxis* titre		Total serum IgE (μg ml^{-1})
		Egg albumen	*N. brasiliensis*	
1	Normal rats no treatment	—	—	most rats < 0.35
2	Primary response to 1 μg EA†	92 ± 36	—	1.33 ± 0.25
3	Secondary response to 10 ng EA	1,228 ± 231	—	1.80 ± 0.39
4a	Potentiated reagin response to EA 12 d after *N. brasiliensis*‡	1,316 ± 270	—	288 ± 47
4b	Not potentiated reagin response to EA 12 d after *N. brasiliensis* (pool of 12 sera)	4,096	—	493
5	Unimmunised rats 12 d after *N. brasiliensis* infection	—	—	247 ± 42
6	Unimmunised rats 18 d after *N. brasiliensis* infection	—	640 ± 109	104 ± 8

*To estimate specific reaginic antibodies by the passive cutaneous anaphylaxis technique[17], 0.1 ml. quantities of saline dilutions of the test sera were injected intradermally in Hooded Lister recipient rats each injection being duplicated on a different animal. 48–72 h later, the animals were injected intravenously with 2.5 mg of EA or 0.5 ml *N. brasiliensis* antigen (saline extract of 500 homogenised worms) together with 0.5 ml of 1% Evans blue which acts as an indicator of vasodilation. The skin reactions were examined after 20 min and the titres recorded above are the greatest dilutions of serum which gave reactions larger than 5 mm in diameter.
†Egg albumen.
‡The method of culture of *N. brasiliensis* and preparation of the infective dose have been previously described[18,19].

immunised with 1 μg egg albumen (Sigma grade V) injected intraperitoneally together with 10^{10} *B. pertussis* organisms (Wellcome Biological Reagents). They were bled again 20 d after immunisation (Group 2) and were then divided into two groups and treated as follows: Group 3 rats received a booster injection of 1 μg egg albumen and were bled 4 d later, while Group 4a rats were infected by subcutaneous inoculation of 4,000 *N. brasiliensis* larvae and were bled 12 d later. These times of bleeding were determined by previous observations of the timing of peak secondary and potentiated reagin responses respectively[6,16].

Table 1 shows the specific reaginic antibody levels and total serum IgE in the serum of these animals. It is evident that the second injection of egg albumen although resulting in a good secondary antigen-specific IgE response (Group 3) did not have a marked effect on total serum IgE. Infection with *N. brasiliensis* on the other hand (Group 4a), as well as causing a similar elevation of egg-albumen specific IgE resulted in a most dramatic elevation of total serum IgE from 1.33–288 μg ml^{-1}. This level is well in excess of that which, by comparison of the egg-albumen reagin titres of the two groups, can be accounted for by egg-albumen reagins alone. Equally the parasite-specific response cannot be implicated at this time after infection since anti-*N. brasiliensis* reagins do not normally appear until later in the infection and no parasite specific reagins could be detected in these areas. A pool of serum from another group of rats with a very marked potentiated reagin response to egg albumen (Group 4b) showed even greater levels of total serum IgE.

The remaining two groups shown in Table 1 were 10 normal animals infected with *N. brasiliensis* without previous immunisation. Group 5 were bled 12 d after infection with 4,000 larvae at which time no parasite specific reagins were present. Greatly elevated levels of total serum IgE occurred in this group in the absence of IgE antibody responses to any known antigens. Group 6 were bled 18 d after infection when parasite specific IgE was present, but when the level of total serum IgE had actually declined.

The results following infection with the parasite *Fasciola hepatica* have been essentially similar to those we report here.

We have also estimated total serum IgE in Wistar rats and find that animals of this strain do not produce levels as high as those of the Hooded Lister strain. For instance, 12 d after *N. brasiliensis* infection the IgE level in Wistar serum (five rats) was 25.3 ± 5.6 μg ml^{-1}. It has been reported that Hooded Lister rats have an exceptional ability to produce IgE antibodies, in that prolonged responses are induced by immunisation with small doses of conventional antigen and that marked secondary responses can occur[16]. It is interesting that this facility is also reflected in very high levels of total serum IgE (up to 493 μg ml^{-1}) following helminth infection in rats of this strain.

The main points which emerge from these results are that infection of the rat with helminth parasites leads to a great elevation of total serum IgE and that the bulk of this IgE is surplus to levels of IgE antibody against known antigens. It would seem reasonable to assume that this IgE consists at least in part of a number of potentiated reagin responses to miscellaneous unknown antigens to which the animal has previously become sensitised. By analogy it is of course tempting to suppose that the high levels of total serum IgE seen in parasite infected human beings consist not exclusively of antiparasite IgE responses to unrelated antigens.

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Alternative route for nitrogen assimilation in higher plants

IT is generally considered that glutamate dehydrogenase (EC.1.4.1.3) is the enzyme that is chiefly responsible for the incorporation of nitrogen into the α-amino group of amino acids:

$$\alpha\text{-oxoglutarate} + NH_3 + NAD(P)H + H^+ = \text{L-glutamate} + H_2O + NAD(P)^+ \quad (1)$$

We suggest that this is not the case in the leaves of higher plants.

There is considerable evidence that chloroplasts are a major site of α-amino nitrogen production[1–5] but, although the chloroplast contains glutamate dehydrogenase, the level of activity is low and the enzyme has a high K_m for ammonia[5], greater in fact than the concentration of ammonia required to uncouple chloroplasts. Chloroplasts also contain glutamine synthetase which has a much lower K_m for ammonia and a much higher activity[4,6,7]. If glutamine synthetase is the route of entry for ammonia, how-

Table 1 Glutamate formation by intact chloroplasts in the presence of various amino donors in the light

Nitrogen donor	Acceptor	Glutamate formed	Glutamine lost
		(μmol per mg chlorophyll h^{-1})	
None	α-oxoglutarate	0.4	—
Glutamine	α-oxoglutarate	6.9	3.6
NH_4Cl	α-oxoglutarate	0.5	—
Asparagine	α-oxoglutarate	3.5	—
Aspartate	α-oxoglutarate	4.8	—
Glutamine	None	0.7	0

The reaction mixture consisted of intact chloroplasts containing about 100 μg chlorophyll, 0.5 μmol α-oxoglutarate and 0.5 μmol of amino donor except asparagine which was 1.25 μmol, in a final volume of 0.3 ml of 0.33 M Sorbitol, 5 mM potassium chloride and 10 mM sodium pyrophosphate, *p*H 7.5.

ever, some way must exist to transfer the amide group of glutamine to the amino group of glutamate. Such an enzyme, glutamate synthase (EC.2.6.1.53), also known as GOGAT, L-glutamine: α-oxoglutarate aminotransferase (NADPH-oxidising) (equation (2)), has been shown to be present in bacteria[8] and yeasts[9], particularly when grown under limiting concentrations of ammonia, and in nitrogen-fixing bacteria and the bacteroids of nodulated higher plants[10,11].

$$\text{L-glutamate} + \alpha\text{-oxoglutarate} + \text{NADPH} + \text{H}^+ = 2\ \text{L-glutamate} + \text{NADP}^+ \quad (2)$$

This pyridine nucleotide-linked enzyme has not been reported in the tissues of higher plants in spite of attempts by ourselves and others[7,11] to find it. Here we present evidence to show that chloroplasts can perform a light-dependent synthesis of glutamate from α-oxoglutarate and glutamine and that extracts of chloroplasts contain a ferredoxin-dependent glutamate synthase.

Chloroplasts from 10–15-d-old *Pisum sativum* (var. Meteor) plants were extracted by a method based on that of Walker[12] in a medium containing 0.33 M sorbitol, 1 mM $MgCl_2$, 2 mM EDTA, 1 mM mercaptoethanol, 10 mM sodium pyrophosphate, *p*H 7.0. The chloroplast pellet was washed in 0.33 M Sorbitol, 5 mM KCl, 10 mM sodium pyrophosphate, *p*H 7.5 and then resuspended in the same medium. Aliquots containing 100–150 μg chlorophyll were incubated with substrates for 20 min at 30° C. The reaction was stopped by the addition of ethanol. Amino acids were separated by paper chromatography in 75% (w/w) phenol, in the presence of ammonia vapour, and quantified by the method of Atfield and Morris[13].

The results (Table 1) show that chloroplasts are capable of converting α-oxoglutarate to glutamate in the light, confirming the findings of earlier workers[14]. They also confirm that this reaction is not stimulated by ammonia—the substrate for glutamate dehydrogenase. When glutamine is supplied, however, there is a seventeenfold stimulation in the rate of glutamate formation and the reaction is absolutely light dependent. The only reaction shown in Table 1 that occurred in the dark was between aspartate and α-oxoglutarate, which was at the same rate as determined in the light. Asparagine also markedly stimulates the conversion of α-oxoglutarate to glutamine. The light dependence of the reaction, compared with the light independence of transaminase activity, and the inability of ammonia to act as an amino group donor would suggest that asparagine is not being cleaved by asparaginase to give aspartate and ammonia which could be subsequently incorporated.

The results with glutamine are best explained by the presence of glutamate synthase. To test this, chloroplasts prepared and washed as described above were resuspended in 0.1 M tricine, *p*H 7.5. After standing for 30 min the ruptured chloroplast debris was centrifuged off and the supernatant passed over a Sephadex G-75 column pre-equilibrated with 0.1 M tricine pH 7.5. The chloroplast extract was eluted with the same buffer. Aliquots of the extract were incubated with various substrates for 20 min at 30° C. The reaction was stopped and the amino acids separated and determined as described above.

Preliminary studies with a spectrophotometric assay confirmed that these extracts had no NADPH dependent glutamate synthase and only a trace of glutamate dehydrogenase activity. But experiments using methyl viologen and dithionite as an electron donating system showed the presence of a glutamine and α-oxoglutarate dependent synthesis of glutamate (Table 2). Subsequent studies showed that ferredoxin is an extremely effective donor; none of the other donors tried was capable of supporting the reaction. The stoichiometry of the reaction is two molecules of glutamate formed for each glutamine utilised, as predicted by equation (2). The rates of glutamate synthesis expressed on a chlorophyll basis are of the same order of magnitude as those in the intact chloroplasts and are also similar to those of nitrite reduction and α-amino nitrogen production measured in other studies[2,4].

The demonstration of a ferredoxin-dependent glutamate synthase in the chloroplasts completes a chain of reactions for the incorporation of nitrate into α-amino nitrogen (Fig. 1). Several considerations suggest this is the major

Table 2 Glutamate formation by chloroplast extracts under various conditions

Electron donor	Nitrogen donor	Acceptor	Glutamate formed	Glutamine lost
			(μmol per mg chlorophyll h^{-1})	
Experiment 1				
Fd + DIT	None	α-oxoglutarate	0*	—
Fd + DIT	Glutamine	α-oxoglutarate	23.2	10.8
Fd + DIT	NH_4Cl	α-oxoglutarate	0	—
Fd + DIT	Asparagine	α-oxoglutarate	0	—
Fd + DIT	Aspartate	α-oxoglutarate	2.08	—
None	Aspartate	α-oxoglutarate	1.55	—
Fd + DIT	Glutamine	None	0	0
Experiment 2				
None	Glutamine	α-oxoglutarate	0	0
Fd + DIT	Glutamine	α-oxoglutarate	9.6	4.9
MV + DIT	Glutamine	α-oxoglutarate	2.8	1.3
NADPH	Glutamine	α-oxoglutarate	0	0
NADH	Glutamine	α-oxoglutarate	0	0
FMN + DIT	Glutamine	α-oxoglutarate	0	0
Fd	Glutamine	α-oxoglutarate	0.5	0.1
DIT	Glutamine	α-oxoglutarate	0.5	0.2

The reaction mixture consisted of 70 μmol tricine, *p*H 7.5, chloroplast extract equivalent to approximately 100 μg chlorophyll, 3.5 μmol α-oxoglutarate and the electron donors and nitrogen donors as indicated. The final volume of the reaction mixture was 0.7 ml. The amounts of electron donors in the reaction mixture was as follows: ferredoxin (Fd) 0.1 mg, sodium dithionite + $NaHCO_3$ (DIT) 1.6 mg each, FMN 0.2 mg, methyl viologen (MV) 0.1 mg, NAD(P)H 0.4 mg. Amino donors were present at 2 mM concentration except asparagine which was at 5 mM. Ferredoxin was derived from *Spirolina maxima*.

*No visible glutamate spot on the chromatogram.

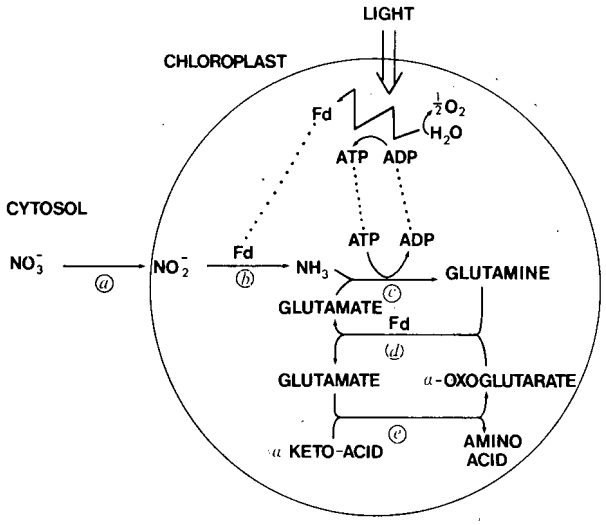

Fig. 1 The proposed route of entry of nitrate into amino acids in leaves. The enzymes are, *a*, nitrate reductase; *b*, nitrite reductase; *c*, glutamine synthetase; *d*, glutamate synthase and *e*, transaminase.

route of entry of nitrogen into the organic form in the leaves of higher plants. The leaf normally receives its inorganic nitrogen in the form of nitrate. This nitrate is metabolised through nitrate and nitrite reductases to ammonia. Both of these reactions are strongly light stimulated[2,3] as is the incorporation of $^{15}NO_3$, $^{15}NO_2$, and $^{15}NH_3$ into α-amino nitrogen[15]. Nitrite reductase is located in the chloroplasts and the presence of glutamine synthetase with a low K_m for ammonia ensures that the ammonia formed can be incorporated without building up to levels which would uncouple the chloroplasts. The presence of glutamate synthase does not preclude a role for glutamate dehydrogenase in ammonia assimilation, particularly under conditions of high ammonia availability. These are most likely to occur in roots. Since ammonia is not normally a major nitrogen constituent of the xylem sap[16] it is unlikely that it occurs in any concentration in the leaves unless it is produced by deamination of transported amines and amino acids.

The operation of the route in Fig. 1 would result in the same pattern of incorporation of the nitrogen of NO_3, glutamate and the amide group of glutamine into all the amino acids. The results of Lewis and Pate[17] with ^{15}N feeding studies confirm that this occurs in leaves. This is distinct from the traditional idea of incorporation of glutamine into relatively few amino acids, as suggested for yeast by Sims and Folkes[18]. The ^{15}N studies of Baker and Thompson[19] with *Chlorella* are also consistent with Fig. 1.

The results we present suggest that asparagine is metabolised by chloroplasts although not by the same enzyme as glutamine. It is probable that the amide is transferred to another acceptor molecule (such as, pyruvate, phosphoenol pyruvate or oxoaloacetate) in the presence of a reductant or to glutamate to form glutamine and pass into the chain. The strong light dependence of glutamate synthesis from asparagine is consistent with the rapid disappearance of asparagine from leaves in the light.

We suggest that the leaves of higher plants contain a glutamate synthase which plays a crucial role in nitrogen assimilation. This enzyme differs from those previously described in being ferredoxin dependent. Its presence helps to explain a major anomaly of intermediary nitrogen metabolism in higher plants, namely the unsuitability of glutamate dehydrogenase as a major point of entry of nitrogen into organic combinations in chloroplasts.

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Note added in proof: Since this paper was submitted we have become aware of a report[20] on NAD(P)H-dependent glutamate synthase type activity in extracts of carrot cell cultures.

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Evolution of cell senescence, atherosclerosis and benign tumours

It has been proposed that cell senescence is the cause of organismic senescence[1–3] and that senescence is under evolutionary control[4]. Orgel[5] has argued that cell senescence could not be under evolutionary control because it is difficult to imagine how senescence of the organism has a positive evolutionary advantage.

I propose that cell senescence itself, rather than senescence of the organism, is the genetically controlled and programmed event selected by evolution and that any effects on somatic senescence is a pleiotropic effect of this function. The advantage of programmed cell senescence to the organism is that it stops cells which have escaped from normal control from dividing indefinitely. If a cell with potential for further division gets out of place in the body, it is possible for it to settle in a new location where the normal environmental signal inhibiting division is lacking. Were these now uninhibited cells capable of unlimited growth, they would divide to fill the entire space available. A limited number of cell divisions, however, would limit the size of the growth, thus saving the organism.

In vitro normal human fibroblasts are limited in the num-

ber of divisions[1,2]. The life of a culture of cells from a tissue explant can be divided into three phases[1]. Phase I ends with the formation of the first monolayer; phase II refers to a period when rapid growth occurs in proper culture conditions, while phase III refers to the period of senescence of the culture. Not only is the percentage of non-dividing cells higher in phase III cells than phase II cells[6], but the growth rate of dividing cells is is reduced[7]: so all cells of the culture are affected by senescence. Phase II cells, however, are unaffected by being mixed with phase III cells[2].

Phase II cells can be held for long periods in a non-dividing state without affecting the number of divisions the cells undergo before senescence[8]. Thus, the metabolic age of a culture can be separated from the doubling age. The onset of senescence is shown to be dependent on the doubling age and the doubling age of a culture can be estimated[9].

Growth of a clone which arose from a single cell is restricted to the edge. The cells in the centre are inhibited by contact inhibition. Thus, the cells in the centre are at a younger divisional age than the cells near the edge[10]. As the cells go out from the centre, they become progressively older and growth stops at the outer edge of the colony when the cells there have divided the maximum number of times and senesce.

The evidence also indicates that cells senesce *in vivo*. With increasing age of the human donor there is a reduction in the number of cell doublings before senescence in culture[11]. Serial transplants of normal tissue into young inbred animals die out[12–14] and the death of the tissue is related to number of cell divisions rather than to its metabolic age[15]. In contrast, transformed cells (malignant cells) which do not show contact inhibition and do not senesce in tissue culture, can be transferred *in vivo* indefinitely[12].

Hayflick postulated that organisms age because their cells age[2,3] and this relationship has been supported by studies on Werner's syndrome, a genetic disease characterised by the development in early adulthood of a wide variety of degenerative features similar to those found in old age. Cells from sufferers of this syndrome are already senescent, doubling less than ten times in tissue culture[11], while cells of normal people of similar age double 35 times. Cell senescence is not necessarily the only cause of organismic senescence since humans aged 70–90 yr are senescent; but fibroblast cultures from them are not senescent, doubling at least twenty times in tissue culture[11], which is over 40% of the doublings found in human embryo cultures.

Atherosclerotic lesions consist of 'plaques' of fibroblasts lining the interior of the great arteries. Limitation in the size of these plaques provides a strong selection for cell senescence.

Benditt and Benditt[16] present the following evidence that a single plaque is monoclonal; that is, all of the cells of a plaque are derived from a single dividing precursor cell. They looked at plaques from the three females who were heterozygous for two electrophoretic variants of the X-linked glucose-6-phosphate dehydrogenase. There is random inactivation of one or the other of the X chromosomes at about the 1,000 cell stage of embryo development, consequently each heterozygous female has a mixture of cells with one or the other X chromosome inactivated[17]. When small pieces (0.1 mm^3) were taken from the aorta wall, both enzyme types were present. But when larger pieces from plaques (0.5 mm^3 to 20–30 mm^3) were taken, most of them had only one isozyme present. It is unlikely that these plaques are scars[18] formed where the artery had been injured, since hypertrophic scar tissue is polyclonal[16].

Cells escaping into the blood stream from points where there is injury to the vascular system would be carried away from the wound by blood flow and could settle on the inner wall of arteries. Possibly they have trouble settling or dividing except where there are fatty streaks, thus giving the correlation between fats and atherosclerosis. The fibroblasts, now living in a new place in the body, exposed to a high velocity flow of nutrient rich blood, would no longer be contact inhibited[19] and would start dividing. As the single cell forms a clone, the cells in the centre away from the flow of blood, will cease dividing, while the cells on the edge will continue to divide. If these cells were able to continue dividing they would form quite a large growth and constrict the artery. Since they are limited to a certain number of divisions, however, the size of the plaque is limited in the same way as the size of a colony arising from a single cell in tissue culture[10].

I am postulating that atherosclerotic plaques arise from cells which escape into the arteries, settle and start growing. To keep these plaques at a minimum size, evolution has selected for a 'clock' which limits the number of cell divisions. This number is set by the conflicting demands of the large number of divisions required for wound repair and cell replacement and the restriction in the number of cell divisions needed to control atherosclerotic plaques and other abnormal growths.

The theory does make several predictions which can be tested. The first is that plaques will grow quickly and then stop when the cells on the outer surface senesce. Second, with fully grown but relatively young plaques, the cells at the centre of the plaque should be able to divide in tissue culture and the ones at the edge should not. Third, the number of divisions the cells from the centre of a plaque can go through should be correlated with the size of the plaque, given that the plaque started from a single cell; and last, the median final size of a plaque should be correlated with the age of the person at the time the cells were released into the blood stream.

Limitation in the size of some benign tumours could also provide strong selection for cell senescence. It has been shown that leiomyomas are monoclonal[20]. Many leiomyomas seem to originate near arteries[21] and thus may have arisen from cells from arterial walls which have escaped into the lyometrium of the uterus. Similar predictions as those made for atherosclerotic plaques apply to such benign tumours. Other benign tumours particularly the large ones, could originate from other causes, such as by a mutation which destroys the system providing contact inhibition. In this case, all the cells, not just the ones on the edge, would go through the maximum number of divisions.

So far no mechanism has been proposed as to how cell senescence takes place. The theory presented above explains how natural selection can select for cell senescence and implies that cell senescence is under genetic control. Views on cell senescence may be divided into two groups: one favours an evolutionary-genetic basis, as in this paper, and the other believes that senescence is caused by random events which create disturbances within the cell which are not fully correctable and that this accumulation of errors eventually leads to death. Any random error theory, such as the 'error catastrophe theory'[5], must be able to explain why cultures of transformed cells are not severely enough affected by the errors to senesce and why normal cells can not evolve the state of perfection enjoyed by transformed cells. Moreover, any random error theory needs to explain why germ line cells are less affected by the errors than the somatic cells. The simplest explanation to these problems is that senescence is under genetic control and that induction of senescence takes place during differentiation leaving the germ line unaffected.

Any mechanism, such as that proposed by the 'error catastrophe theory', could be used by evolution as the means to induce senescence. But a special compound could be produced whose sole function is to induce senescence.

Milo[22] has presented evidence that a glycoprotein extracted from the cell wall of senescent phase III cells causes almost immediate senescence when put on phase II cells.

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Insulin stimulates myogenesis in a rat myoblast line

THE clonal rat myoblast cell line L_6 isolated by Yaffe[1] undergoes myogenesis when the mononucleated myoblasts become confluent and fuse into multinucleated myotubes. This morphological differentiation is correlated with the synthesis of characteristic muscle proteins such as myosin and creatine kinase[2]. When L_6 myoblasts fuse the specific activity of adenylate cyclase[3] decreases and cyclic AMP, dibutyryl cyclic AMP and theophylline reversibly inhibit the differentiation process[4]. In primary cultures of rat muscle cells, a decrease in cyclic AMP has been reported at the time of fusion[3]. These facts support the hypothesis that low levels of cyclic AMP correlate with myoblast differentiation, while high levels inhibit fusion. In view of the relationship between the action of insulin and the biosynthesis of cyclic nucleotides[6] and the effects of the hormone on muscle metabolism[7], we have examined the influence of insulin on myogenesis of cultured L_6 myoblasts. The hormone replaces partially the serum requirement for morphological differentiation of primary cultures of chick embryo myoblasts[8,9], and now we have found that it stimulates fusion and the specific activity of creatine kinase in L_6 myoblasts in the presence or absence of serum.

In an attempt to separate the effects of insulin on myogenesis from those on metabolism, we examined the effect of the hormone on myoblast fusion in the presence of different concentrations of serum (Fig. 1). In serum-free medium almost no myotubes were observed in the absence of insulin and many cells died (Fig. 1*a*). In 1% serum, myotubes formed after 2 d and in 10% serum they appeared after 4 d (Fig. 1*c* and *e*). Addition of insulin (1 μg ml^{-1}) to the medium stimulated both the rate and extent of fusion at all serum concentrations tested (Fig. 1*b*, *d* and *f*), most of the nuclei being incorporated into myotubes within 24 h of the onset of fusion. Myotubes formed in the presence of insulin were much larger than those formed in its absence,

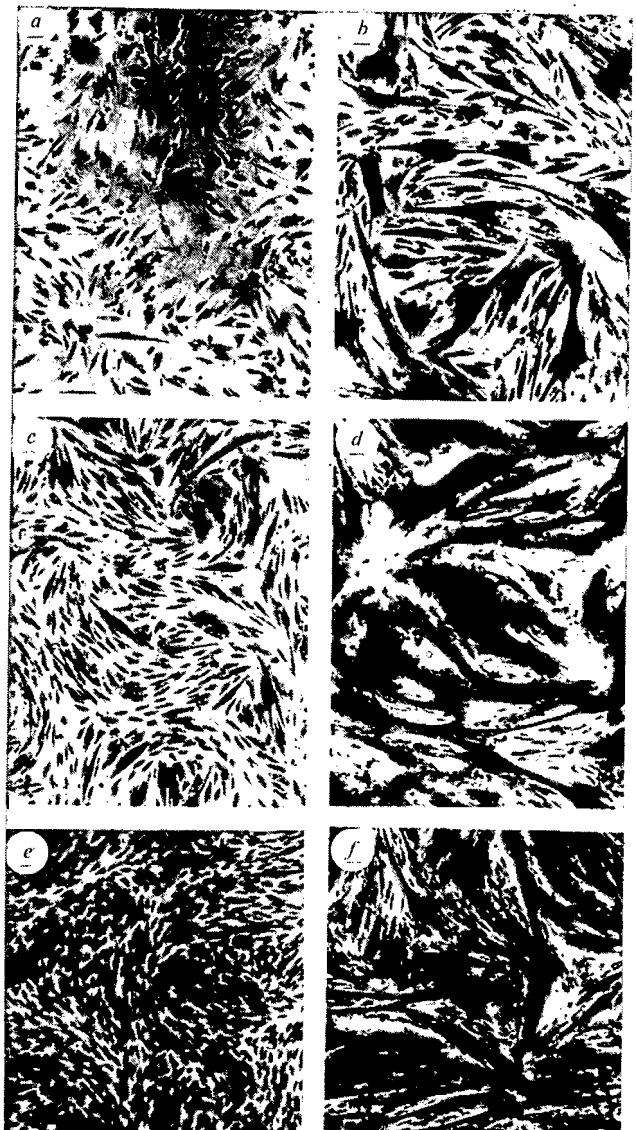

Fig. 1 Effect of insulin and serum on myoblast fusion. Rat myoblast L_6 cells (from Dr S. Heinemann, Salk Institute) were grown at 37° C in a humidified incubator under a 5% CO_2 95% air atmosphere in complete medium (Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum, Grand Island Biological Co.). To study fusion, cells were plated in complete medium at 8×10^3 cell cm^{-2} in 35 mm dishes. This medium was removed 24 h later, the dishes were rinsed with phosphate-buffered saline, and re-incubated under the following conditions. *a* and *b*, Serum-free medium containing 1.5 mg ml^{-1} bovine serum albumin (BSA); *c* and *d*, medium containing 1% foetal calf serum; *e* and *f*, complete medium (10% foetal calf serum). Insulin (1 μg ml^{-1}, zinc-free, from Dr C. Yip, University of Toronto) was added when the medium was changed for *b*, *d*, and *f* and the dishes were stained with methylene blue 3 d later.

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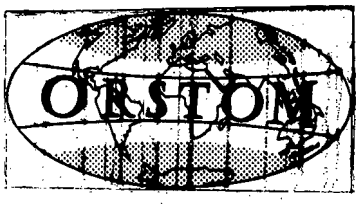

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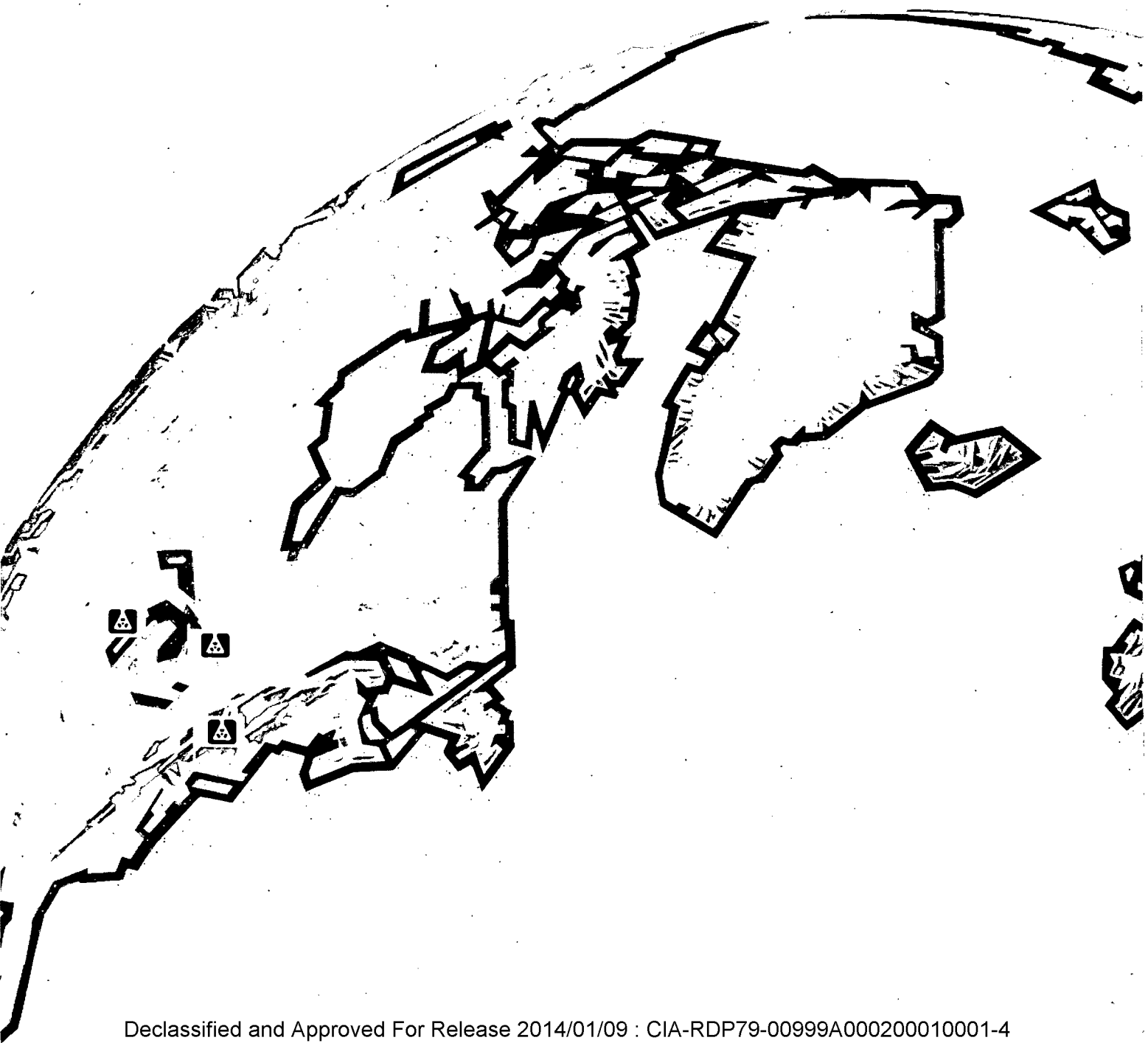

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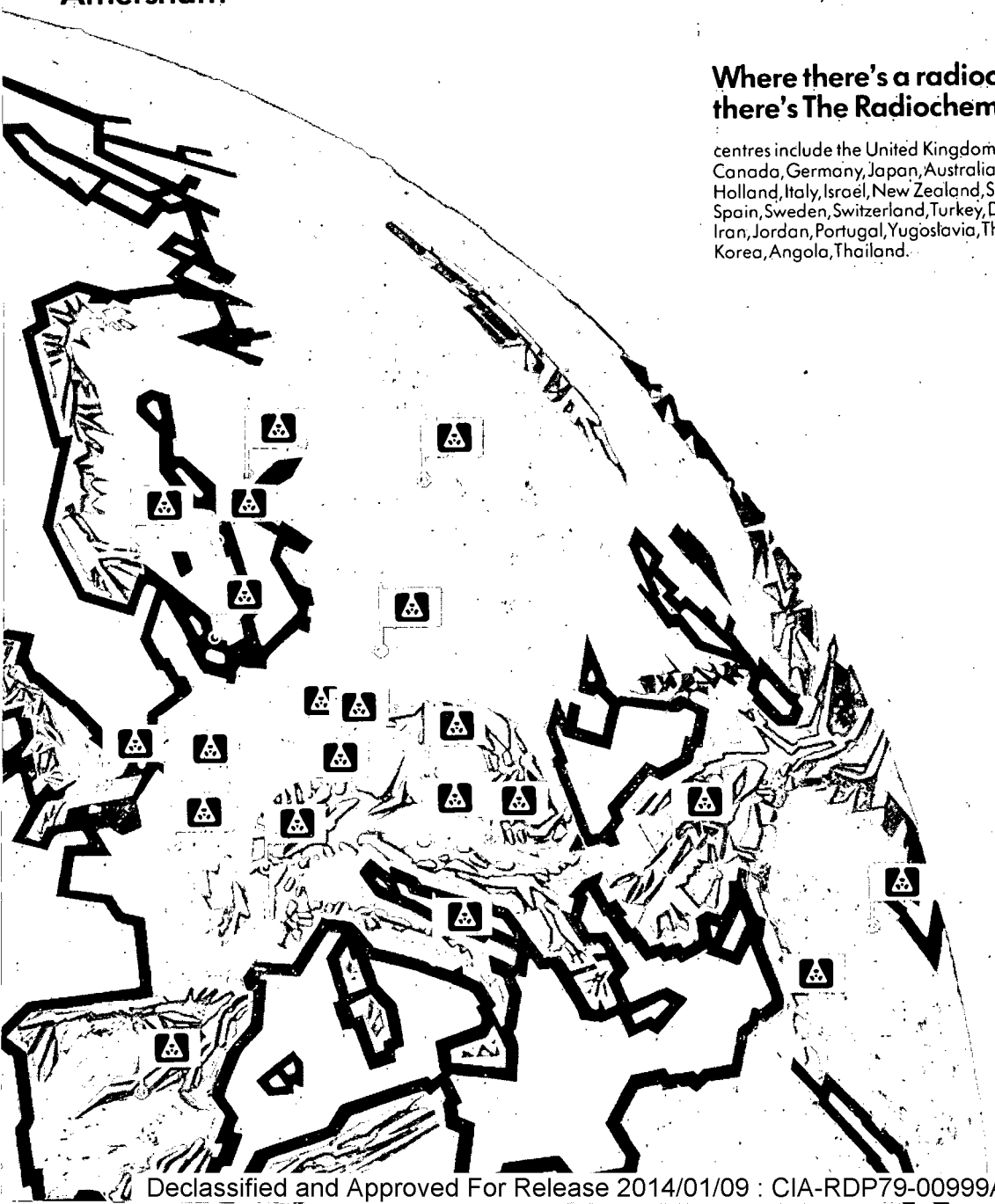

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with nuclei located more centrally. They also tended to degenerate more rapidly[8].

We then found that in the presence of insulin the specific activity of creatine kinase increased markedly at three serum concentrations (Fig. 2). The increase coincided with the onset of fusion (data not shown). The highest specific activity was reached in cultures grown in 1% serum in the presence of insulin, and under these conditions the maximum was reached in only 48 h (Fig. 2*b*). The increase in creatine kinase activity (as well as in morphological fusion) was delayed by about 24 h in cultures containing 10% serum (Fig. 2*c*). By changing the insulin-containing medium at 4 d, we obtained a further stimulation of creatine kinase levels in the presence of 10% serum, but not 1% serum (dashed lines, Fig. 2*b* and *c*). In other experiments the glycogen phosphorylase content of myotubes, detected histochemically[2], also increased after addition of insulin. It is interesting that de la Haba *et al.* found no accumulation of the characteristic muscle enzyme glycogen synthetase in primary cultures of myoblasts fused in the presence of insulin in serum-free medium[9]. Since under similar conditions with the L_6 myoblast line we observed a marked stimulation of creatine kinase activity by insulin, either there are differences in the response to insulin of primary cultures and L_6 cells, or glycogen synthetase and creatine kinase levels are controlled independently during myogenesis.

All the above experiments were carried out using 1 μg ml^{-1} of insulin. When we used 10^{-2} μg ml^{-1} insulin (Fig. 3*a*) we observed a significant stimulation of morphological fusion and creatine kinase activity: this concentration is about one order of magnitude higher than that found *in vivo*. When the concentration of serum was low, the maximum stimulation was obtained with 1 μg ml^{-1} insulin, while in 10% serum no plateau was apparent even with 5 μg ml^{-1} insulin. The requirement for high concentrations of insulin for maximum stimulation of creatine kinase may be due in part to inactivation of the insulin, especially in 10% serum (compare Fig. 2*c*). It may also indicate that a continuous and prolonged exposure to insulin is needed for the increase in fusion and creatine kinase activity. This possibility was tested by exposing cultures to insulin for various times, up to 4 d, removing the hormone, then measuring creatine kinase activity at 4 d. Figure 3*b* shows that insulin had to be present for more than 24 h to stimulate significantly creatine kinase specific activity, and longer exposure resulted in greater enzyme activity.

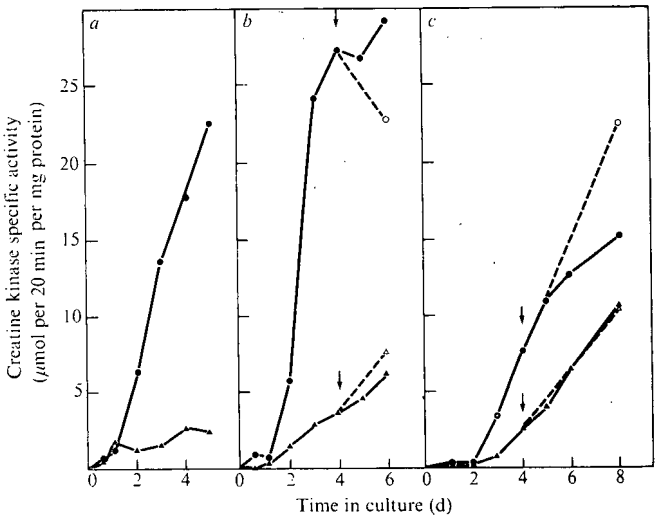

Fig. 2 Effect of insulin and serum on specific activity of creatine kinase. Cells were plated as described in the legend to Fig. 1, and the medium was replaced by medium containing various concentrations of serum in the presence or absence of insulin (1 μg ml^{-1}). At the indicated times after the medium change, the medium was removed, the cells were rinsed quickly with buffer *A* (100 mM Tris-acetate, *p*H 6.8 (22° C), 25 mM magnesium acetate, 10%, v/v, glycerol), drained, and kept frozen at −35° C. After thawing, cells were scraped into 0.35 ml buffer *A* supplemented with 0.2%, v/v, Nonidet P-40, and 0.1 mM phenymethylsulphonylfluoride (Sigma) and subjected to two cycles of freezing and thawing. Aliquots from duplicate plates were assayed for creatine kinase[10] and protein[11]. Variations in specific activity were usually less than 15% for values greater than 1 μmol per 20 min per mg protein. *a*, Serum-free medium containing 1.5 mg ml^{-1} BSA; *b*, medium containing 1% foetal calf serum; *c*, complete medium (10% foetal calf serum). ▲, Minus insulin; ●, 1 μg ml^{-1} insulin. The open symbols indicate values obtained after replacing the medium on day 4 (arrows) with fresh medium with (○), or without insulin (△).

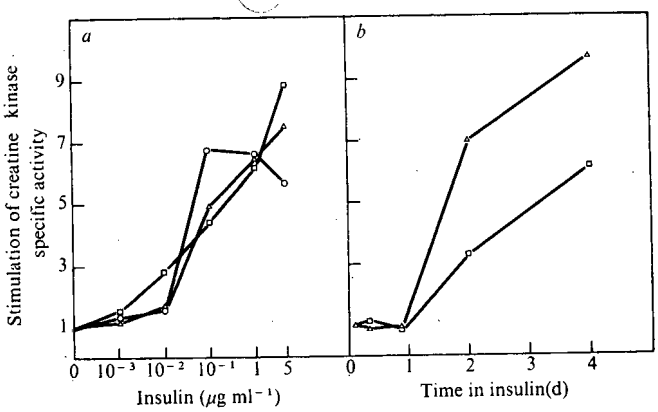

Fig. 3 Characteristics of the stimulation of creatine kinase by insulin. Cells were handled as described in the legend to Fig. 1, insulin was added at the time of the medium change, and cells were frozen 4 d later for determination of specific activities as described in the legend to Fig. 2. Results are expressed as relative stimulation over the control incubated without insulin. *a*, Insulin concentration dependence. Control specific activities were 1.0, 1.0 and 0.6 μmol per 20 min per mg protein in 0, 1% and 10% serum respectively. *b*, Dependence on time of exposure to insulin. Insulin (1 μg ml^{-1}) was added at time 0 for various times after which the medium was removed and replaced by medium at the same serum concentrations but lacking insulin. Initial specific activities without insulin were 1.3 and 0.6 μmol per 20 min per mg protein in 1% and 10% serum respectively. ○, No serum; △, 1% serum; □, 10% serum.

Insulin stimulates many cellular activities, especially in muscle cells[7], and its observed effect on myogenesis in both L_6 cells and primary cultures[8] may not be a specific effect on differentiation, but an indirect result of a general stimulation of cellular metabolism, especially in serum-free medium. For example, in fibroblasts serum starvation results in a reduction in RNA and protein synthesis, and these activities can be restored by the addition of insulin[12]. In the case of L_6 myoblasts, however, cell density and protein synthesis are higher in 10% serum minus insulin than in serum-free medium plus insulin, yet myogenesis is increased in the latter case. (We have also found that insulin does not increase RNA or protein synthesis in the first 30 h after its addition to cells grown in 10% serum; a twofold stimulation was obtained later at 48 h during an 8 h label period.) Thus, although further experiments are needed, we believe it unlikely that the stimulation of the specific activity of creatine kinase and the morphological changes caused by insulin are due to nonspecific stimulation of protein synthesis.

Alternatively, the insulin stimulation of myogenesis may be a consequence of alterations in cyclic nucleotide metabolism, since insulin is known to affect cyclic AMP synthesis[13] and degradation[14] and cyclic GMP levels[6] in different cell types. Therefore it is attractive to suppose that such effects account for the stimulation of myogenesis in L_6 myoblasts reported here, and perhaps for the insulin-promoted differentiation of mammary gland in organ

culture[15], and of primary cultures of lens epithelium[16]. In the case of L_6 myoblasts, it is worth noting that insulin improves both the reproducibility and synchrony of fusion, and allows myogenesis to be studied in a defined serum-free medium.

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Sickle cell resistance to *in vivo* hypoxia

THE rapid disappearance from circulation of human erythrocytes transfused to rats can be prevented if the animals are pretreated with ethyl palmitate (EP) and a cobra venom factor (CVF)[1]. The improvement in human erythrocyte survival in these animals is due to EP-induced impairment of reticuloendothelial function[2] and to suppression of intravascular haemolysis through CVF inhibition of the third component of complement[3]. The halflife of normal human erythrocytes in the blood of untreated rats is less than 15 min, but is about 30 h in the EP and CVF treated rats[4].

Application of this animal system to the study of human sickle cell anaemia erythroctyes (sickle cells) has verified many of their known survival characteristics in man[5–7]. These include a short intravascular survival, an improved survival with *in vivo* hyperoxygenation and an improved survival following *in vitro* cyanate treatment[8,9]. Human sickle cells transfused into rats exposed to widely divergent concentrations of oxygen seem to consist of two populations with regard to their *in vivo* sensitivity to oxygen changes. The circulation of a minor population of sickle cells is dependent on the concentration of oxygen the rats breathe, as shown by rapid increases and decreases in circulating cells with increases and decreases in the amount of oxygen administered to the rats (O_2-sensitive cells)[8,10]. In contrast, the majority of sickle cells seems to be relatively insensitive to the levels of hypoxia attainable *in vivo*. They continue to circulate after the initial rapid removal of the oxygen-sensitive cells in spite of exposure to hypoxic atmospheres barely compatible with life. The proportion of sickle cells which is oxygen sensitive is not influenced by the total number of sickle cells transfused (O.C., S.C.F., and G.O., unpublished). Oxygen-sensitive sickle cells are most readily demonstrated when the animals breathe 100% O_2 followed by 7–8% O_2 in short alternating periods. In these conditions the ^{51}Cr survival curve of the transfused sickle cells assumes a characteristic 'saw tooth' pattern with peaks during hyperoxia and valleys during hypoxia[10].

Here we report that separation of hypoxia-resistant sickle cells is possible if blood from a rat previously transfused with sickle cells is removed during hypoxia and then retransfused into another rat. Presumably all of the human sickle cells in circulation in the blood of donor animals during hypoxia are insensitive to a low pO_2 after the sensitive population has been sequestered. Figure 1 shows that in the recipient animals the

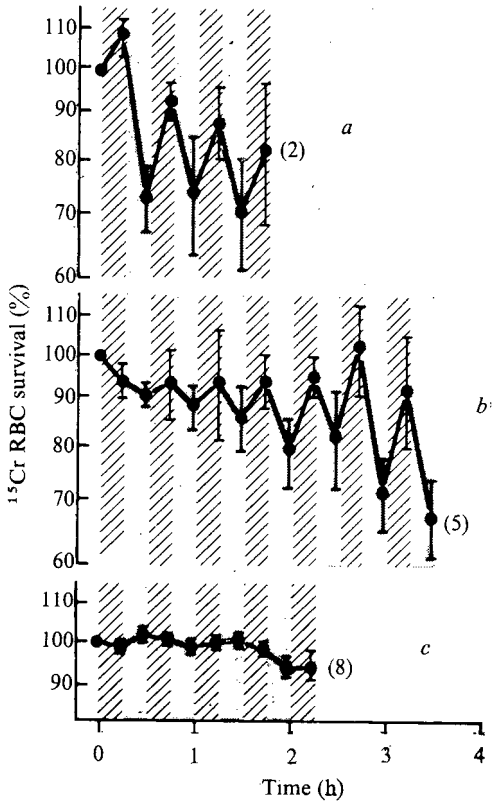

Fig. 1 Human sickle cell resistance to O_2-dependent circulatory changes after their removal from hypoxic rats and retransfusion into separate animals. Each rat was injected with 0.5 g kg^{-1} of EP and with 10 units of CVF 2 h before transfusion. Three donor animals then were transfused with 4 ml of a 50% saline suspension of ^{51}Cr-labelled erythrocytes (RBCs) from one of three patients with sickle cell anaemia. After a 20–60 min exposure of the donor animals to 7.5% O_2 in N_2 5 ml of rat blood—containing transfused ^{51}Cr human sickle cells—were removed by cardiac puncture. The RBCs were washed with physiological saline, adjusted to a 50% suspension in saline and then 1–3 ml were retransfused into recipient animals. Following this, all rats were exposed to a minimum of three hyperoxia–hypoxia cycles each consisting of 15 min of 100% O_2 followed by 15 min of 7.5% O_2 in N_2. The ordinate measures ^{51}Cr RBC survival expressed as % activity found in the rats' blood immediately before exposure to the hyperoxia–hypoxia cycles. The abscissa measures time (h) after start of the first hyperoxia–hypoxia cycle. Points in the graph represent means and bars ± 1 s.d. of the values for each patient study. The number of rats used in each group is shown in parentheses. *a*, Transfused sickle cells showing that the sickle cells remaining in the two donor animals that survived cardiac puncture exhibited characteristic O_2-dependent circulation during each hyperoxia–hypoxia exposure. *b*, Retransfused sickle cells (previously obtained from hypoxic donor animals) did not show this behaviour initially. After the third cycle, an O_2-sensitive population developed in the retransfused sickle cells. *c*, The survival in the rats' blood of ^{51}Cr-labelled control human RBCs obtained from two individuals without haematological abnormalities was not influenced by oxygen changes. Stippled areas, hypoxia; cross-hatched areas; hyperoxia.

survival curves of retransfused sickle cells obtained from hypoxic donor animals do not exhibit the saw tooth pattern during the initial alternating periods of hyperoxia and hypoxia. The sickle cells remaining in the donor animal (transfused sickle cells) continue to show this response. Sickle cells removed from donor animals while they breathe 100% O_2 and retransfused into recipient animals (not shown in the figure) retain an O_2 sensitive population. Continuation of the hyperoxia–hypoxia cycles in the recipient rats with O_2 insensitive retransfused sickle cells eventually results in a survival pattern consistent with the development of a population of sickle cells which is oxygen sensitive (Fig. 1). The proportion of O_2-sensitive sickle cells to total sickle cells is similar to that in the original population. Wide changes in O_2 tension seem to be necessary for the appearance of an O_2-sensitive population of sickle cells in the recipient animals. This statement is based on the observation (O.C., S.C.F., and G.O., unpublished) that the O_2-insensitive retransfused sickle cells in rats maintained on room air will remain resistant to O_2 changes until they have been subjected to several hyperoxia–hypoxia cycles.

The physical separation of hypoxia resistant sickle cells from the total population confirms the heterogeneity of human sickle cells with regard to their oxygen dependent circulation in this animal model. The temporary loss of O_2-sensitive sickle cells from the animals' circulation during exposure to low O_2 tension is best explained by *in vivo* sickling and trapping of sickled erythrocytes in the animals' microvasculature. This explanation is supported by our finding in this animal model of hypoxia sensitive erythrocytes not only from patients with sickle cell anaemia but also from patients with sickle-thalassaemia and with sickle-Hb C disease[4]. In contrast, none of eight individuals with sickle trait studied thus far has shown this type of response[11].

The observations reported here demonstrate that a proportion of O_2-insensitive sickle cells can be transformed into an oxygen sensitive population when challenged with repeated hyperoxia–hypoxia cycles. Our findings suggest that repeated sickling may be an important factor in the production of cells that do not circulate during hypoxia. Direct extrapolation of animal data to the human situation must be viewed with caution. Nevertheless, it is possible that vaso-occlusive episodes in patients with sickle cell anaemia may be a function of the relative number of sickle cells that are oxygen sensitive with regard to their capacity to circulate. The isolation and study of any oxygen resistant population may help to determine those factors that protect sickle cells from sequestration during *in vivo* hypoxia.

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Expression of the dystrophia muscularis (*dy*) recessive gene in mice

STUDIES *in vitro* of mutant gene expression in murine muscular dystrophy now seem to have reached an impasse. For 15 years there was only one species of dystrophic mouse[1], the Bar Harbor 129 ReJ strain (denoted dy/dy). Then in 1970, a second strain BH WK ReJ (denoted dy^{2J}/dy^{2J}) was reported[2] which was described as being similar in all histopathological respects to the allelic dy/dy mutant except that it showed a slower progression of muscle necrosis. The new mutant gene was transferred to another mouse strain C57Bl/6J for commercial retail. The new strain, offering many advantages over the original strain, has been readily adopted for study by research workers; certain findings obtained with this strain have added confusion to a difficult problem simply because it is not the same as the original strain.

Over the past four years I have been growing dystrophic (dy/dy) muscle *in vitro*, and have noticed that muscle explants from crush lesions in dystrophic (dy/dy) muscle did not form multinucleate myotubes, whereas muscle explants from crush lesions in normal muscle readily did so. I report here some brief details of this work (previously described in refs 3 and 4), and include the results of some preliminary experiments using dystrophic (dy^{2J}/dy^{J2}) mice which suggest that allelic mutant genes do not have the same phenotypic expression.

Crush lesions were made in the triceps and quadriceps muscles of normal Bar Harbor 129 ReJ+/+, dystrophic 129 ReJ dy/dy, and dystrophic C57Bl/6J dy^{2J}/dy^{2J} mice under pentobarbital anaesthesia. Four days after the lesion was inflicted the mouse was killed by cervical dislocation and the lesion zone was removed under sterile conditions for tissue culture. The excised regions were dealt with separately. The tissue was chopped and the explants set up in coded Pulvertaft[5] chambers or 50-mm Petri dishes. The culture medium was 10% EE50, 10% horse serum and 80% Eagle's MEM with streptomycin and penicillin.

Each day, over a period of 8 d, all the coded cultures were assessed microscopically using differential interference optics. The assessment was carried out blind and the results are therefore unbiased. The total number of cultures was in excess of 100 for normal and dy/dy, and 40 for dy^{2J}/dy^{2J} mice.

Days 1, 2 and 3 showed essentially the same pattern of growth in all cultures. Proliferation and explant spreading were similar, and occasional cells with up to five nuclei were found. From day 4 to day 8 proliferation continued until the cultures became overgrown and degenerated. During this second period all normal cultures (Fig. 1*a*) and all dystrophic dy^{2J}/dy^{2J} cultures (Fig. 1*b*, *d*) developed multinucleate myotubes of considerable size. There was an indication, which could not be quantified, that explants of dy^{2J}/dy^{2J} quadriceps showed less multinucleation than triceps cultures from the same animal. All dystophic dy/dy cultures (Fig. 1*c*) showed associations of cells which have been named pseudostraps[4]. These are muscle strap-like or myotube-like aggregates of myoblasts which do not fuse. There were no multinucleate structures of any kind to be found. I believe that these results clearly demonstrate an abnormality of early myogenesis caused by the expression of the dy/dy gene, but no indication of any abnormality caused by the expression of the dy^{2J}/dy^{2J} gene.

The results clarify some of the conflicting evidence already produced. Three groups[6–8] have used organotypic methods of tissue culture combining foetal spinal cord sections with adult muscle. Paul *et al.*[7] were very careful not to draw any far-reaching conclusions from their work, recognising the genetic background on which it was based. But they did state that adult myogenic cells were in some way affected by the dystrophy (dy/dy) and were incapable of

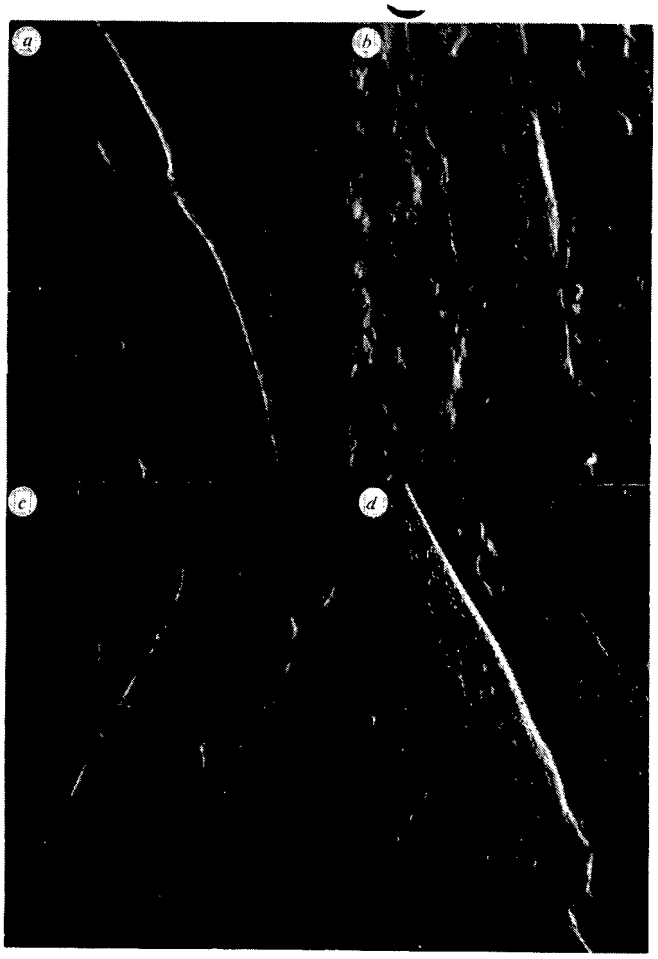

Fig. 1 Unstained, living cultures seen using differential interference optics (Reichert). They are representative of muscle formation *in vitro* from crush lesion explants. *a*, Part of a large multinucleate myotube showing a chain of central nuclei (arrows), normal +/+. *b*, Part of a myotube from dystrophic dy^{2J}/dy^{2J} quadriceps. c, Pseudostraps from dystrophic *dy/dy* muscle. Note the close associations of cells which always remain discrete. *d*, A clear multinucleate myotube from a dystrophic dy^{2J}/dy^{2J} triceps culture. All photographs were taken at 7 d *in vitro*, under identical conditions.

regeneration. Hamburgh *et al.*[8] came to the conclusion that muscle from dystrophic (dy^{2J}/dy^{2J}) mice exhibited a capacity to regenerate similar to normal muscle. Gallup *et al.*[6], using both strains of mice, concluded that the regeneration of normal and dystrophic muscle is dependent on the genotype of the cord section with which it is coupled. Peterson[9], using mouse chimeras (SWV+C57Bl/6J dy^{2J}/dy^{2J}), was led to suggest that the presence of dystrophic nuclei did not affect myogenesis in any way. My results are essentially similar to those of Paul *et al.*[7] and Hamburgh *et al.*[8] I cannot, however, agree with any of the findings or conclusions of Gallup *et al.*[6]. Finally, I am in agreement with Peterson[9] that dy^{2J}/dy^{2J} muscle behaves in the same way as normal muscle with regard to myogenesis.

Therefore I would submit that the recent study of murine dystrophy has been based on the false assumption that allelic mutant genes have the same phenotypic expression. Consequently, if researchers into muscular dystrophy are to continue to use the mouse as an experimental animal they must take care to specify the strain of animal used before drawing up conclusions relating to the pathogenesis. These findings suggest that the causal lesion of the *dy/dy* dystrophic mutant lies in the muscle cell; this seems unlikely to be the case in the so-called (dy^{2J}/dy^{2J}) dystrophic strain.

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Growth of human muscle spindles *in vitro*

STRIKING histological, histochemical and physiological differences between intrafusal and extrafusal muscle have been observed in a large number of species. Enzyme profiles, for instance, of intrafusal muscle not only fail to reflect the extrafusal pattern but show considerable interspecies variation[1,2].

The precise relationship of genetic, neural and environmental influences and their role in determining the peculiar properties of intrafusal muscle, is uncertain. There is strong evidence that innervation is essential for the normal ontogenetic development of spindles[3] and neural control of the histochemical properties of fully differentiated extrafusal muscle has been shown to be considerable[4]. Spindles denervated at maturity, however, are remarkably resistant to change.

We considered that an investigation of the growth and differentiation of intrafusal muscle *in vitro*, thus isolated from its normal neural and environmental influences, might elucidate the role of genetic factors in determining its characteristics *in vivo*. It was of particular interest to examine the behaviour of human tissue in this context in view of the large number of genetically determined muscle disorders and the relative lack of information concerning the pathological role of the spindle.

The regenerative capacity of skeletal muscle following injury, disease, or experimentally-induced trauma is considerable[5,8] and is, to some extent, reflected by the growth and differentiation of muscle explants in culture. Normal and diseased adult human muscle has been successfully grown *in vitro* by several workers[7,9], but none has specifically investigated the growth of intrafusal muscle. Whether spindles possess a similar regenerative potential and to what extent intrafusal muscle retains its identity in the simplified conditions of tissue culture is as yet unknown.

The varied findings of other workers in the field indicate that the culture employed may itself influence results and, for valid comparisons to be made between the behaviour of different tissues *in vitro*, must be a controlled factor. For this reason, in our study, explants of both extrafusal and intrafusal muscle from each specimen were cultured in identical conditions.

Fig. 1 A multinucleate myoblast from a culture of intrafusal muscle after 36 d *in vitro* (×280.80).

Muscle specimens (commonly of palmaris longus) were obtained from patients undergoing diagnostic, motor-point muscle biopsy. Adjacent specimens in each case were examined by routine histological and histochemical procedures and cases in which the results of these investigations were found to be within normal limits were used in this study.

The specimen, approximately 2 cm×0.5 cm was placed in a Petri dish containing Eagles minimum essential medium (MEM) with 100 U ml^{-1} penicillin and streptomycin. Any spindles present were dissected out from the surrounding tissue under a microscope, care being taken to isolate the spindle completely from the extrafusal fibres. The outer layers of the spindle capsule were removed and the spindle cut transversally to form explants approximately 1 mm long. The explants were placed on coverslips coated with reconstituted rat tail collagen and covered with a thin clot of chicken plasma. They were then placed in test tubes with 3 ml of a nutrient medium consisting of 80% MEM, 10% horse serum and 10% chick embryo extract. Each tube was sealed with a silicone rubber bung, placed on a tube roller and incubated at 37° C. Small pieces of extrafusal muscle (approximately 1 mm cube) were set up in a similar manner. The medium was changed once a week and living cultures were examined from time to time using the Maximow slide system and phase contrast microscopy. All the above procedures were carried out aseptically. At intervals between 20 and 70 d after setting up, suitable cultures were fixed and stained with PTAH.

Fifteen stained cultures of normal intrafusal muscle have so far been obtained, these being derived from nine different patients and representing less than half the number of normal intrafusal cultures initially set up, the remainder have failed to grow. In all cases parallel extrafusal cultures were also obtained.

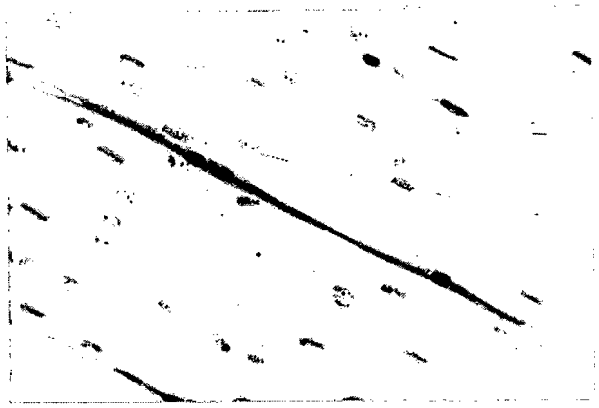

Fig. 2 A myotube from a culture of intrafusal muscle after 55 d *in vitro* (×176.80).

The first growth of intrafusal muscle is observed between 10 and 25 d *in vitro* and may precede signs of growth in parallel extrafusal cultures by several days. Microscopic examination at this early stage reveals, however, only mononucleate cells and it is likely that a large proportion of these are fibroblasts derived from the relatively large amount of capsular connective tissue inevitably left within the explant.

Immediately following the initial proliferation of mononucleate cells, typically elongated and spindle-shaped in appearance, the majority of cultures show in addition a variable number of multinucleate cells. Figure 1 shows a multinucleate myoblast in an intrafusal culture at 36 d; such cells are visible within the first few days of growth following the lag period and continue to be found in cultures 70 d old. The cells show extreme variability of size, shape and number of nuclei and in older cultures there is, in addition, an increasing proportion of myotube forms, elongated syncitia in which some or all of the nuclei become separated or aligned in chains (Fig. 2). At a later stage peripherally situated nuclei are seen in many of these cells.

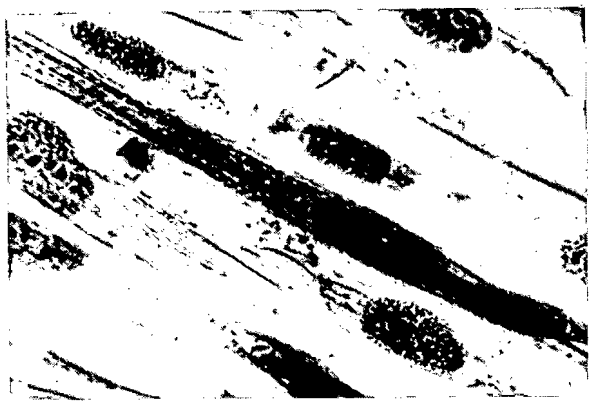

Fig. 3 Cross striations in a myoblast from a culture of intrafusal muscle after 70 d *in vitro* (×728).

Cross striations were observed in a small minority of myoblasts and myotubes in both intrafusal and extrafusal cultures (Fig. 3). They were seen in both mononucleate and multinucleate cells and when fully differentiated Z lines, A and I bands and occasionally H zones were visible, but at no time during the observation of any of our living cultures was spontaneous contraction observed.

Owing to the small number of cultures so far obtained and the widely varying appearance of the growing cells, statistical examination of results has not yet been undertaken, though some measurements of size have been made. In a culture of intrafusal muscle at 31 d in which the lag period was 20 d (that is, after 11 d growth) myotubes reached a maximum length of 720 μm. This value became 800 μm in another culture after 18 d growth. (These cells were less than 20 μm in width.) Another intrafusal culture after 35 d growth produced many shorter, broader myoblasts, commonly 100 μm long and 50–60 μm wide, containing numerous densely packed nuclei. The longest cell reached only 400 μm.

In parallel extrafusal cultures, cells also showed extreme variation of form, but in all cases measured, the maximum length of myotube exceeded the value for intrafusal cultures, for example up to 1,100 μm after 18 d growth. This observation, however, may only reflect the considerably larger number of extrafusal explants obtainable from any specimen and their relatively greater mass.

It is apparent that intrafusal muscle possesses a considerable regenerative capacity under tissue culture conditions

and there is little doubt that regeneration of muscle is a function of satellite cells, of which there are many within muscle spindles[9]. No ultrastructural differences can be discerned between extrafusal and intrafusal satellite cells and our preliminary observations indicate that the growth and differentiation of intrafusal muscle *in vitro* closely parallels the behaviour of extrafusal muscle. If confirmed by further investigation, for instance of the histochemical and ultrastructural properties of the growing cells, it would suggest that a critical factor for the differentiation of muscle spindles is trophic rather than genetic and is absent under these conditions. A strong possibility is that such a factor derives from the neural influence and it is interesting to consider to what extent this could be supplied in a tissue culture system and how much its precise nature may be analysed by this means.

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Multiple control mechanisms underlie initiation of growth in animal cells

THE understanding of the control of cell proliferation and differentiation requires the unravelling of the early events that trigger the initiation of growth. An *in vitro* system to study the initiation of growth is provided by the mouse 3T3 cell line. These cells exist in two alternative growth states: either reversibly arrested in the G_0 phase of the cell cycle or in active proliferation. When resting 3T3 cells are exposed to fresh serum they recommence DNA synthesis and cell division[1]. Functional membrane changes are among the earliest events associated with the reinitiation of growth[2]. Within minutes of serum addition, the rate of transport of inorganic phosphate, nucleosides and glucose is increased several fold[2–6], while cyclic AMP, which has been implicated in the regulation of growth of cultured fibroblasts, shows an opposite change[7–11].

The coordinated expression of these membrane changes raises the possibility that some of these events are cause-effect related to each other[12,13]. Two different hypothesis concerning this important problem have evolved. A unifying view holds that cyclic AMP acts as a pleiotypic modulator that regulates the overall rates of all the membrane transport systems affected by changes in cell growth[5,14]. The alternative view proposes that cyclic AMP might control some specific changes in membrane transport but not a general change in cell permeability [10,11]. The first hypothesis predicts that there should be a high degree of coupling between the various biochemical changes stimulated by serum or insulin while the second envisages the changes as dissociable, since they are supposed to be controlled by several distinct mechanisms.

The experiments presented here were conducted to differentiate between these alternative possibilities. Our results substantially support the hypothesis of specific changes and suggest that at least three distinct mechanisms of control of membrane transport operate when growth is initiated.

Uridine and phosphate transport systems respond to separate control mechanisms as shown by the differential effects produced by the addition of ouabain, cycloheximide and cyclic AMP elevating agents. Typical experiments illustrating this point are shown in Fig. 1. Ouabain, an

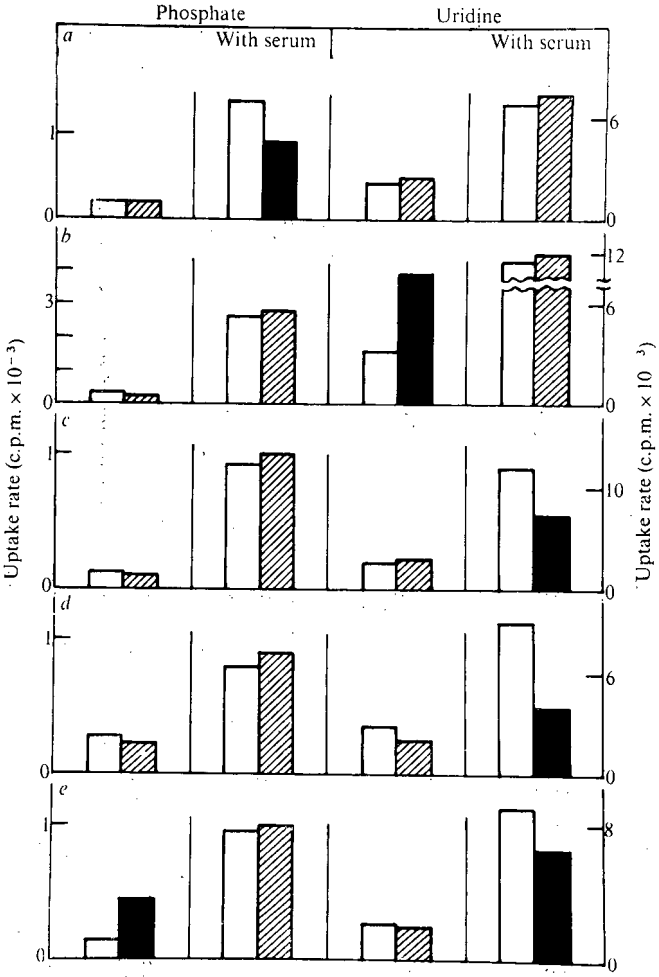

Fig. 1 Differential effects of, *a*, 1 mM ouabain; *b*, 10 μg ml^{-1} cycloheximide; *c*, 1.5 mM theophylline; *d*, 0.2 mM SQ 20006 and *e*, 40 μg ml^{-1} PGE_1 on uridine and phosphate uptake rates. Swiss 3T3 fibroblasts were maintained in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum, 100 units ml^{-1} penicillin and 100 mg ml^{-1} streptomycin in Nunc Petri dishes. Transport was measured on cells attached to 30 mm dishes. Confluent cultures were used usually 3 d after the last change of medium. First, the cells were washed with phosphate-free medium and incubated in this medium with (hatched bars) or without the above mentioned compounds at the concentrations indicated. After 10 min, dialysed foetal calf serum (10% final concentration) was added to some dishes and the culture incubated for additional 20 min. Then, the cells were labelled with ^{32}P-phosphate (75 μCi μmol^{-1}) 3.75 μCi ml^{-1}) and ^{3}H-uridine (29 mCi μmol^{-1}, 2.5 μCi ml^{-1}) for 5 min. Uptake into acid-soluble pools and all other materials and methods were measured as described previously[10–11]. Each bar represents the average of two transport determinations. Each experiment was repeated at least three times. The treatments that produced effects were shown with dark bars.

inhibitor of the (Na^+, K^+) APTase, significantly decreases the activation of phosphate transport produced by serum but has no effect either on the increase of uridine uptake or on cyclic AMP levels. Conversely, elevation of the intracellular levels of cyclic AMP by prostaglandin E_1 (PGE_1), theophylline or 1-ethyl-4-hydrazino-1H-pyrazolo-(3,4-b)-pyridine-5-carboxylic acid, ethyl ester, hydrochloride (SQ 20006) (ref. 15), depresses the activation of uridine transport by serum while the stimulation of phosphate uptake is unaffected. In the absence of serum, PGE_1 stimulates the uptake of phosphate but not that of uridine. Further, cycloheximide added in the absence of serum activates uridine uptake, in agreement with earlier results[5,12] but it does not modify the basal uptake level of phosphate. In addition, when the uridine transport rates determined in the presence of different compounds are plotted as a function of cyclic AMP levels measured in parallel cultures, an inverse correlation is obtained. On the other hand, no correlation is obtained when phosphate transport rates are plotted as a function of cyclic AMP concentration (data not shown). All these results show that the early transport changes of uridine and phosphate are dissociable.

In addition to stimulating phosphate and uridine transport, serum brings about a large increase in 2-deoxyglucose uptake in resting 3T3 cells[4-6]. A third level of transport control was identified when this phenomenon was carefully examined. The activation curve as a function of time is clearly biphasic (Fig. 2). The early phase was unaffected by the presence of cycloheximide while the second was completely prevented by this inhibitor. Other experiments reveal that the kinetic parameter modified as a function of time is the maximum velocity (L. J. de A., and E. R., unpublished). Thus, in addition to changing rapidly the uptake of 2-deoxyglucose, serum seems to induce also the synthesis of new carrier molecules. For comparison, uridine and phosphate transport rates were measured in similar conditions (Fig. 2). A biphasic activation curve with a cycloheximide-sensitive second phase was also seen with phosphate uptake but not with uridine up to 5 h after serum addition.

The induction of the second phase of glucose transport is not triggered by a drop in cyclic AMP since SQ 20006 does not block it (Table 1) and insulin which decreased cyclic AMP in resting cells[7,8,10] and increases cyclic GMP in different tissues[16] does not induce it. Cyclic AMP, however, could modulate the activity of the transport system when it is already inserted in the membrane (Table 1) which may account for the rapid phase stimulated by serum.

The possibility of eliciting or inhibiting selectively some early biochemical events by using a variety of compounds clearly shows that these membrane changes are dissociable. All these results support the hypothesis that conceives distinct mechanisms of control switched on when growth is initiated and seem incompatible with a pleiotypic role for cyclic AMP in the control of all the changes in membrane transport produced by serum. In fact from the present and previous findings[10,11] it is possible to define at least three distinct mechanisms of control, that can be differentiated on the basis of temporal expression, dependence on cyclic AMP levels and on (Na^+, K^+) ATPase activity and requirements for protein synthesis.

The various metabolic changes studied here might be produced independently by different factors present in serum or by different chemical signals synthesised by the cells when exposed to a single factor. Another important question that can now be asked is whether or not all the biochemical changes stimulated by serum are required for the initiation of cell division. Our studies offer experimental basis for attacking these problems, the clarification of which will contribute substantially to our understanding of the initiation of growth.

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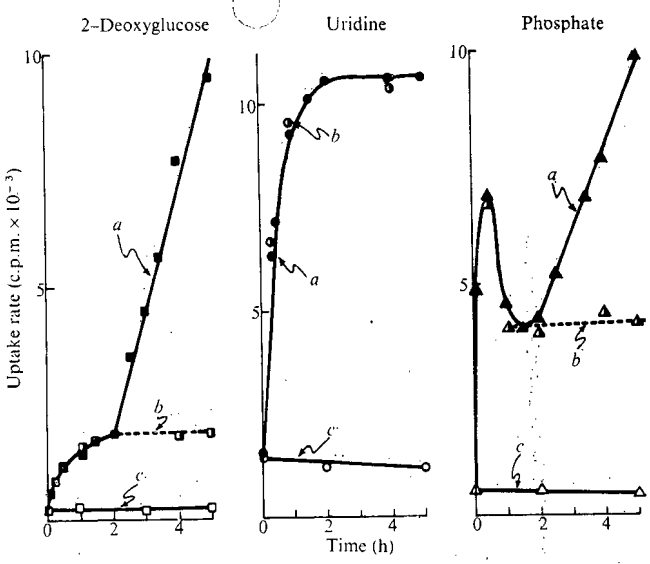

Fig. 2 Kinetics of the changes in 2-deoxyglucose, uridine and phosphate transport produced by the addition of fresh serum to resting cultures of 3T3 cells in the absence or presence of cycloheximide (10 μg ml^{-1}). The inhibitor was added at time 0. The uptake of ^{3}H-2-deoxyglucose was determined as follows: the cells were washed four times with prewarmed serum-free medium minus glucose and incubated for 10 min in this medium with or without dialysed serum or other additions. The cells were then exposed to labelled substrate (3 μCi ml^{-1}; 5×10^{-5} M) for 10 min. Uptake into the 5% TCA soluble fraction was linear for 15 min and was measured as described earlier[10,11]. The other determinations were carried out as indicated in the legend to Fig. 1. *a*, Serum; *b*, serum + cycloheximide; *c*, serum-free medium.

Table 1 Effect of SQ 20006 on the uptake of 2-deoxyglucose stimulated by serum*

	Additions		Uptake rate
	Serum	SQ 20006	(c.p.m. per dish)
A	−	−	320
	+	−	890
	+	+†	520
B	−	−	280
	+	−	7,751
	+	+†	4,256
	+	+‡	5,179
	+	+§	7,002

* Resting cultures of 3T3 cells were exposed to 15% foetal calf serum for 40 min (A) or 4 h 30 min (B). Then, the uptake of 2-deoxyglucose was measured as described in the legend to Fig. 3.
† Added at time 0.
‡ Added 4 h after serum.
§ Added at time 0 and removed 4 h later by washing four times with prewarmed medium.

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Control of cell division in yeast using the ionophore, A23187 with calcium and magnesium

A23187 is an ionophore which exhibits specificity for divalent cations and promotes their passive transport across biological membranes[1]. It therefore effects equilibration of divalent ions between the cell sap and the surrounding medium and counteracts any active accumulation of ions within the cell. Evidence, mainly from studies on sea urchin eggs, supports the hypothesis that calcium ions play an important part in bringing about cell division[2-4]. We have found that division in the fission yeast, *Schizosaccharomyces pombe*, is associated with a doubling in magnesium concentration within the cells[5]. It, therefore, seems likely that, if accumulation of divalent ions within the cell were an essential prerequisite of cell division, this should be inhibited by A23187 and cell division should not take place, and so we have investigated this possibility.

S. pombe, strain 132, was grown in the defined medium, EMM2 (ref. 6). Once the cells were growing exponentially and had reached a concentration of 10^6 cells ml^{-1} or more, 1.0 ml of the stock solution of A23187 was thoroughly mixed with 100 ml of culture. The stock solution was prepared by dissolving 0.5 mg A23187 in 0.05 ml of Analar acetone and adding 1 ml of absolute ethanol[7]. Subsequently, cell division was inhibited quite rapidly, only 10–15% of the cells dividing before inhibition was complete. The ethanol/acetone mixture on its own had no effect.

If cells which had been left in growth medium with A23187 as above for 3 h at 30° C were collected and immediately inoculated into fresh medium, synchronous division (cell plate index = 25–30%) ensued about 30 to 40 min after transfer. The next division was also synchronous. DNA measurements showed that the cells had been inhibited by A23187 in the G2 phase of the cycle. Following staining with Giemsa[8], up to 47% of the cells appeared to be binucleate. Cells with a complete cell plate, but in which division had not started as indicated by formation of a 'waist' at the cell plate, were taken as one cell. Thus, slightly more than half of the binucleate cells contained cell plates. Occasionally a cell was observed containing two cell plates close together and, in between, a piece of cytoplasm undergoing autolysis.

To define more precisely the point at which inhibition of division was occurring, a synchronous culture of *S. pombe* was established using selection of newly divided cells from a sucrose gradient[6]. Samples were taken from this culture at suitable intervals and A23187 added to give the same final concentration as above. These samples were left for 3 h at 30° C and the cell number measured. In the absence of inhibition, division should have occurred within this time. This was verified using controls without A23187 but with the ethanol/acetone solvent. Even with a sample of cells taken only 10 min before division could be detected, A23187 completely prevented division taking place. Thus the block to cell division must occur very close to the end of the cell cycle. Possibly the initial blockage is at a stage just before nuclear division, whereas any cell at a point after nuclear division and before cell division is blocked at other stages which are particularly sensitive to the changes brought about by the ionophore.

Table 1 Change in calcium and magnesium content of cells following addition of 5 μg ml^{-1} A23187 and incubation at 30° C for 3 h

	Ca (g per cell)	Mg (g per cell)
Before addition of A23187		
Supernatant liquid	3.0×10^{-14}	0.5×10^{-14}
Pellet	37×10^{-14}	1.3×10^{-14}
After addition of A23187 and incubation		
Supernatant liquid	5.0×10^{-14}	0.9×10^{-14}
Pellet	5.0×10^{-14}	0.6×10^{-14}

Since A23187 is supposed to make membranes permeable to divalent ions, it was decided to investigate in general terms what happened to calcium and magnesium within the cell. Samples of cells were taken before and after A23187 treatment in EMM2 for 3 h as described above. These samples were quickly washed twice in glass-distilled water and homogenised in 5 ml glass-distilled water in an Eaton press[9]. The homogenate was centrifuged at 40,000*g* (R_{av}) for 30 min. The supernatant liquid was analysed without further treatment, using atomic absorption spectrophotometry to measure calcium and magnesium. The pellet was solubilised by digestion overnight at 40° C with 3 ml of a 1:2 (v/v) mixture of 60% perchloric acid and 30% hydrogen peroxide. The resultant solution was diluted to 10 ml with glass-distilled water and analysed for calcium and magnesium in the same way as the supernatant. The results obtained are shown in Table 1. It is clear that, following A23187 treatment, calcium in the pellet has dropped dramatically (sevenfold) while magnesium has dropped by half. The supernatant values have increased, but to a lesser extent. Since the pellet almost certainly contains mitochondrial vesicles, and there is evidence from other eukaryotic cells that mitochondria accumulate calcium and, to a must lesser extent, magnesium[10], this may reflect leakage of ions from the mitochondria brought about by A23187. The supernatant levels would then reflect altered steady state concentrations in the cytosol. Overall, the total calcium content of the treated cells has dropped to 25% of that of untreated cells while the magnesium content has dropped to 80%. This probably reflects the differential gradients for these ions between the cell and the medium where the calcium concentration is 0.07 mM and the magnesium concentration 2.5 mM.

Finally, we attempted to reverse the effects of A23187 by adding calcium and magnesium to cells which had been treated with A23187 in EMM2 as previously described for 3 h. Increasing the calcium concentration of the medium tenfold (to 0.7 mM) and the magnesium concentration likewise (to 25 mM) enabled division of about 90% of the cells to occur between 15 and 20 min after adding the ions as their chloride salts. This is somewhat faster than the recovery when the cells are transferred to fresh medium (see above) but no further divisions ensue whereas the cells in fresh medium recover completely.

Similar results to the above have been obtained for the budding yeast, *Kluyveromyces fragilis* (Penman, unpublished). We therefore conclude that a pool of calcium and magnesium is essential for cell division in yeast. It may be required to promote the breakdown of microtubules involved in nuclear

division and cell plate formation[11] and this possibility is being investigated.

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Antigen of mouse bile capillaries and cuticle of intestinal mucosa

CELL surface antigens have been isolated by solubilisation and fractionation of membranes[1-3]. Here we present data on the membrane antigens of mouse liver cells soluble in the nonionic detergent Triton X-100.

Mouse liver cell ghosts were obtained as in ref. 4. Triton fractions (TF) from ghosts and from whole mouse liver cells were obtained as in ref. 5. TFs were also prepared from mouse kidney, intestine, spleen and the mouse ascitic hepatoma 22-a.

Liver TFs were analysed by gel double diffusion[6] and by immunoelectrophoresis[7] using rabbit antisera to liver cell ghosts. These sera do not react with soluble antigens of the liver cell and in immunofluorescent studies they stain predominantly the plasma membranes of parenchymal cells[8] (Fig. 1*a*). Before analysis all the TFs were concentrated 10–20 times and anti-ghost sera were concentrated four times. Each TF of mouse liver was checked by the precipitin reaction with 40 samples of rabbit anti-ghost sera obtained from different animals and in different cycles of immunisation.

In all TFs of mouse liver cells or ghosts studied, six antigens were found which were precipitated by the analysed rabbit anti-ghost sera. These antigens were presumably membrane antigens. One of them, called here BCCA (bile capillary-cuticular antigen) was regularly found in each liver TF whereas the other five antigens were detected in only some of the TFs. The latter five antigens are probably present in too small amounts to be detected consistently. It seems however, that modifications in the method of preparation of TF will eventually overcome these variations. Using immunoprecipitation and indirect immunoautoradiography the distribution of BCCA in different mouse organs and in the ascitic hepatoma 22-a was studied. BCCA is the dominant antigenic component of the TFs from the liver cells and ghosts and it is stable during storage. It is detected in considerable amounts in TFs of the mouse kidney. In TFs of the ascitic hepatoma 22-a it is determined only by immunoautoradiography. It is not detected in concentrated saline extracts of mouse liver, kidney, spleen or intestine by either the agar precipitation test or immunoautoradiography.

BCCA was partly purified by agar-gel preparative electrophoresis[7]. The combined and concentrated TFs of the mouse liver were electrophoresed in buffered 1% agar (0.5 N barbital buffer, *p*H 8.6). The electrophoretic fractions (in the region of β_1-globulin) with maximal BCCA concentration and minimal amounts of other antigens were collected and combined.

This partially purified BCCA and the anti-ghost serum containing antibodies predominantly to BCCA were used for the preparation of monospecific antibodies to BCCA[10].

The cell localisation of the BCCA was studied by indirect immunofluorescence. The acetone-fixed cryostat sections of mouse organs were treated with monospecific antibodies to the BCCA and then by FITC-labelled purified donkey antibodies to rabbit IgG. The specificity of the fluorescence was checked by fluorescence inhibition.

Distinct, bright fluorescence was found on the liver cell surface surrounding the bile capillaries between two hepatocytes (Fig. 1*b*). The Kupfer cells, the epithelium of the bile duct and the vascular endothelium were not stained. The fluorescence of bile capillaries disappeared after the absorption of the monospecific antibodies to the BCCA with the BCCA-containing electrophoretic fraction. The native anti-ghost serum used for the preparation of the BCCA monospecific antibodies also selectively lacked the ability to stain bile capillaries after the absorption with the electrophoretic fraction containing BCCA (Fig. 1*c*).

In the sections of intestine (Fig. 1*d*) the monospecific antibodies to the BCCA stained the cuticle of the mucosa epithelial cells of villi and crypts. This distinct cuticular fluorescence, at different intensities, was observed for the whole length of the intestine from the duodenum to the rectum. The other morphological structures in the sections of the intestine were not stained. The cuticular fluorescence disappeared after the absorption of the monospecific antibodies to BCCA with TFs of the liver or intestine.

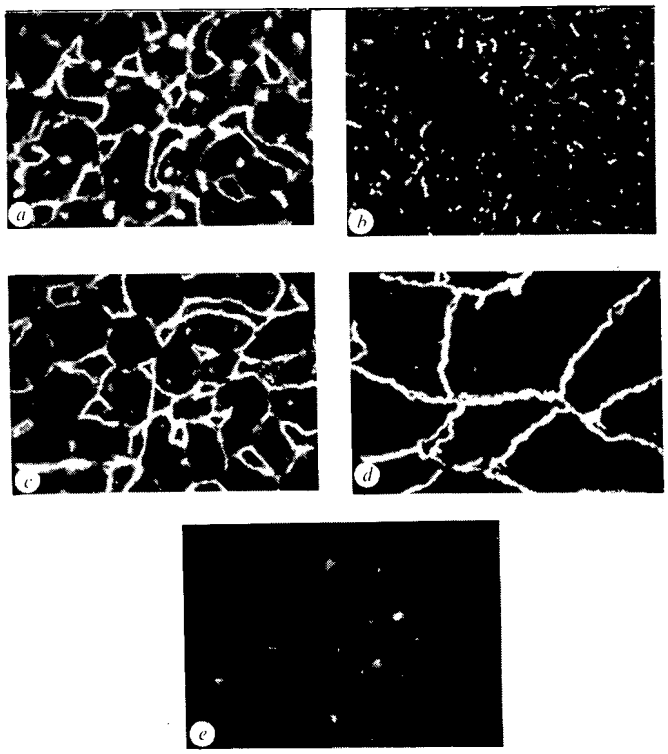

Fig. 1 *a*, Mouse liver section treated with native antiserum to liver cell ghosts. × 162. *b*, Mouse liver sections treated with monospecific antibodies to the BCCA. × 72. *c*, Mouse liver section treated with antiserum to liver cell ghosts absorbed by the partially purified BCCA (electrophoretic fraction). × 162. *d*, Sections of mouse intestine and *e*, stomach treated with monospecific antibodies to BCCA. × 162.

The monospecific antibodies to BCCA did not stain sections of the mouse oesophagus, stomach, kidney, spleen, pancreas and sections of the solid variant of the mouse ascitic hepatoma 22-a (the ascitic hepatoma, inoculated subcutaneously) (Fig. 1*e*).

So, BCCA is specifically localised in the bile capillaries of the mouse liver and the cuticle of the intestinal mucosa epithelial cells. A small amount of BCCA (or an antigen with a similar determinant) is also found on the plasma membrane of each liver cell. The absence of BCCA in the cytoplasm of liver cells suggests that it is synthesised on the cell surface.

The specific antigen of rat liver bile capillaries was described by Sulitzeanu *et al.*[12]. They also found it in kidney proximal tubule cells. Another antigen of rat bile capillaries has been reported[13]. The authors considered this antigen to be organo-specific but they did not demonstrate its absence in the intestine and other rat organs.

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Ultrastructural analysis of toxin binding and entry into mammalian cells

VERY low concentrations of phytotoxins can kill mammalian cells, and some of them, such as ricin and abrin, isolated from *Ricinus communis* and *Abrus precatorius*, have been reported to suppress protein synthesis in cells [1–3] and cell-free systems[4,5]. Ricin inhibits peptide chain elongation by catalytically inactivating the 60S subunits of ribosomes[5–7]. It is a galactose-binding glycoprotein of molecular weight 60,000, containing two nonidentical subunits linked by disulphide bridges[8–11] and seems to be identical to the lectin *R. communis* agglutinin II (RCA_{II}) isolated by affinity chromatography[8–12]. Ricin, or RCA_{II}, has been used to suppress the growth of ascites tumour cells *in vivo*[13], and as a cell surface probe for oligosaccharide receptors[14–17] containing terminal D-galactose or N-acetyl-D-galactosamine-like saccharides[11,14,18,19]. I have found that RCA_{II} enters mammalian cells by endocytosis (similar to the findings of Oliver *et al.*[20]) and is then released into the cytoplasm, where it apparently acts directly on protein synthesis.

Although ^{125}I-labelled RCA_{II} binds rapidly to mammalian cell surfaces[14,21] (saturation < 10 min at 5° C)[14], there is a significant lag before protein synthesis is inhibited (> 30 min at 37° C)[21,22]. This suggests that events subsequent to cell binding are essential for RCA_{II} toxicity[21,22]. Also, the toxic action of RCA_{II} can be prevented by addition of lactose[21] or antitoxin[22] within 15–30 min. In cell-free systems RCA_{II} suppresses protein synthesis almost immediately (≈1–5 min)[4,6,21], and this action is not sensitive to RCA_{II} saccharide inhibitors[21]. To investigate the ultrastructural events subsequent to cell binding I have used an electron-dense ferritin-conjugate of RCA_{II} (Fer-RCA_{II})[23,24].

Fer-RCA_{II} was synthesised as described before[23–25] and purified by affinity chromatography on Biogel A-1.5 m[24]. After elution of column-bound Fer-RCA_{II} by 0.2 M D-galactose, the conjugate was dialysed extensively. Affinity purified Fer-RCA_{II} was active in suppressing murine 3T3 fibroblast protein synthesis, and the inactivation required much more time than required for simple binding to the cell surface (Table 1). The Fer-RCA_{II} conjugate was not as active in suppressing protein synthesis as native RCA_{II}; this might reflect some inactivation during the coupling process or a steric interference of ferritin with the enzymatic properties of RCA_{II}. But, as with RCA_{II}, the conjugate was blocked by the addition of D-galactose to the incubation medium (Table 1). To study the ultrastructural interaction of Fer-RCA_{II} with cells, the conjugate was added to 0.02% EDTA-dissociated, washed, confluently grown murine 3T3 cells suspended in Dulbecco's modified Eagle's minimal essential medium[26] (DMEM) containing 5% calf serum for various times at 4°, 20° or 37° C. Alternatively, cells were pulsed with Fer-RCA_{II} for 5–15 min at 4° C, washed by centrifugation and suspended in DMEM and then incubated further at 37° C for different times. Controls were labelled identically escept that 0.1M D-galactose was present in the labelling and wash solutions. In some of the samples D-galactose was added at specific times during Fer-RCA_{II} labelling. Incubations were terminated by washing the cells in fresh DMEM and then adding 1.5% glutaraldehyde in sodium phosphate-buffered saline (*p*H 7.2; 310 mosmol). Fixation proceeded for at least 1 h, the first 30 min at the incubation temperature. The cells were washed again and postfixed in 1% osmium tetroxide in 0.1 M sodium phosphate buffer, *p*H 7.2, for 1 h at 20° C, washed, dehydrated in ethanol-propylene oxide and embedded in Epon 812. Thin sections were stained with 1% uranyl acetate and observed in a Hitachi HU-12 electron microscope.

3T3 cells labelled with Fer-RCA_{II} for 5–30 min at 0°–4° C or 5–10 min at 20°–37° C before fixation showed continuous labelling of RCA_{II} receptors, exclusively at the cell surface (Fig. 1*a*). Labelling was specific as D-galactose inhibits conjugate binding (Fig. 1*b*), as previously demonstrated[24]. Labelling at higher temperatures (20°–37° C) resulted in eventual lectin-induced receptor clustering of some but not all RCA_{II} receptors and subsequent endocytosis. At 37° C this sequence of events was more rapid than at 20° C, and significant endocytosis was seen within 60 min (Fig. 1*c*). With times greater than 60 min at 37° C most Fer-RCA_{II} molecules were localised inside intact or broken endocytotic vesicles and free in the cell cytoplasm (Fig. 1*d*).

To determine the sequence of Fer-RCA_{II}-receptor interaction and endocytosis, pulse labelling experiments were performed. Cells were labelled with Fer-RCA_{II} for 5–10 min at 0°–4° C, washed by centrifugation, and resuspended in DMEM at 37° C for various times. To duplicate samples, D-galactose was added immediately after the Fer-RCA_{II} pulse at 0°–4° C, or after 5, 15, 30 and 60 min subsequent incubation at 37° C. After the 5 or 10 min pulse of Fer-RCA_{II} at 0°–4° C, the ferritin-lectin molecules located at the cell periphery could be completely or almost completely removed from the cell surface by addition of D-galactose (0.05 M final). After shifting Fer-RCA_{II}-labelled cells to 37° C following labelling and then washing at 0°–4° C, progressively less Fer-RCA_{II} could be removed from cells with time by addition of D-galactose. Also, the location of Fer-RCA_{II} changed from exclusively extracellular in a dispersed distribution (0–5 min after shifting to 37° C), to both extracellular and intracellular (clustered at the

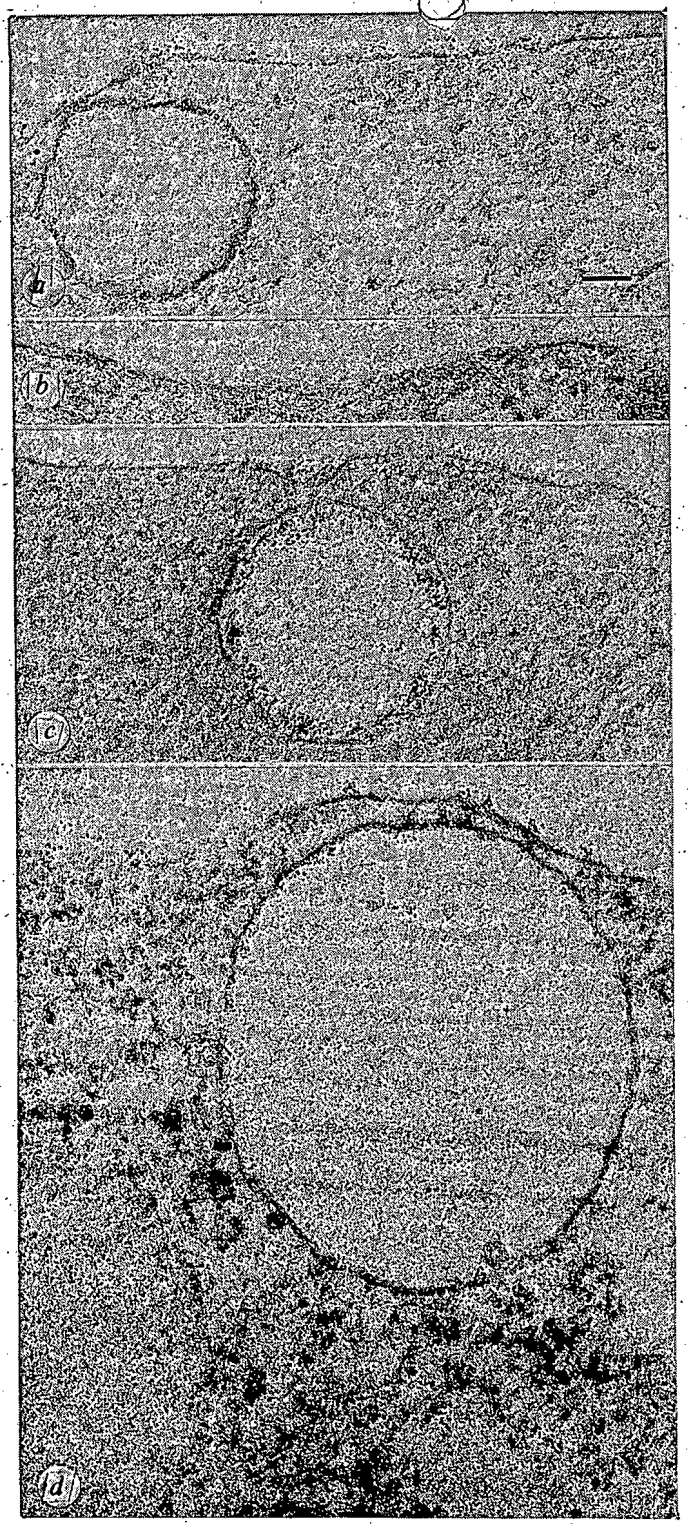

Fig. 1 Fer-RCA_{II} binding to murine 3T3 cells at 37° C. *a*, After a 10 min incubation, ferritin was localised exclusively at the cell surface more or less randomly distributed. Note that the vesicle to the left is unlabelled. *b*, Labelling is specific as D-galactose abolished Fer-RCA_{II} binding activity. *c*, Between 30 and 60 min of incubation Fer-RCA_{II} clustered at the cell surface and endocytosis has begun. *d*, After 60–90 min incubation ferritin was localised inside the cell in intact or broken endocytotic vesicles and free in the cell cytoplasm. All magnifications 68,000; bars equal 0.1 μm.

cell surface and also inside endocytotic vesicles) 30–60 min after shifting to 37° C, to predominantly intracellular (inside endocytotic vesicles and free in the cell cytoplasm) at times greater than 60 min after shifting to 37° C. Generally, the

Table 1 Inhibition of cell protein synthesis by ferritin-conjugated *Ricinus communis II agglutinin*

Pretreatment* Additions	Time (min)	Incorporation of ^{3}H-Leu into protein c.p.m.†
None	0	111,700
‡RCA_{II}	0	102,100
	10	103,200
	60	2,400
‡RCA_{II} + §D-galactose	60	91,400
¶Fer-RCA_{II}	0	105,100
	10	103,300
	60	5,100
¶Fer-RCA_{II} + §D-galactose	60	85,700

* Confluently grown 3T3 cells were removed from substrate with 0.02% EDTA in TC-PBS and washed twice in DMEM containing 5% calf serum. The cells were adjusted to a concentration of 5×10^6 per ml and 0.5 ml aliquots were incubated with or without Fer-RCA_{II} (~100 μg ml^{-1} total protein) for various times in DMEM at 37° C in a CO_2 incubator.

† Cells were treated with Fer-RCA_{II} and washed in DMEM minus leucine and incubated in DMEM plus ^{3}H-leucine (3 μC ml^{-1}) for 60 min at 37° C in a CO_2 incubator. The cells were washed three times in TC-PBS and dissolved in 1 ml of 1M sodium hydroxide containing 0.1% bovine serum albumin. Protein was precipitated by addition of 4–5 ml cold 10% trichloroacetic acid. After a 60 min incubation at 4° C, the contents of each tube were filtered through Whatman G F/C glass filters with vacuum. The filters were washed twice with 5% trichloroacetic acid and once with 95% ethanol and dried. The dried filters were placed in scintillation fluid and counted using a Beckman Model LS200 scintillation counter.

‡ Final concentration, 1 μg ml^{-1}

§ Final concentration, 0.05 M

¶ Final concentration (total protein), approximately 100 μg ml^{-1}

Fer-RCA_{II} that had moved into cells by endocytosis was not susceptible to removal by addition of D-galactose.

Results consistent with these have been obtained with ^{125}I-RCA_{II} (ref. 21). After a 10 min pulse (1 μg ml^{-1} ^{125}I-RCA_{II}) at 4° C, approximately 95% of the cell-bound ^{125}I-RCA_{II} could be removed by lactose, but less than 20% could be removed after a 60 min subsequent incubation at 37° C (ref. 21). In parallel experiments less than 5% inhibition of protein synthesis was obtained after the 10 min pulse of RCA_{II} at 0°–4° C. By 30 min after shifting to 37° C, inhibition was approximately 10–20%, and by 60–90 min, it was >80%, similar to the results obtained with ferritin-RCA_{II} (Table 1). In cell-free protein synthesis systems inhibition was almost complete within 1–2 min under these conditions[21]. These studies also stress the importance of performing quantitative lectin labelling experiments for short times at low temperatures to preclude endocytosis of the surface bound lectin molecules[14-16,27,28].

Refsnes *et al.*[22] proposed a two-step model for the toxic action of abrin[15,22,29] and ricin on mammalian cells: in the first step the toxin binds to the cell surface, and in the second step it (or portion of it) is transported into the cytoplasm. My results are consistent with this model, and further suggest that lectin-induced surface aggregation[30-33] and endocytosis[20,21,22] are important in the entry of toxin into mammalian cells. The ultrastructural fate of Fer-RCA_{II} after endocytosis suggests that the lectin molecules are released from endocytotic vesicles, perhaps by breakdown. Lysosomes do not appear to be involved in this process, because I did not observe fusion of lysosomes with the endocytotic vesicles; however, this cannot be ruled out for all vesicles. The fact that simple RCA_{II} binding to cells is not sufficient to cause inhibition of protein synthesis suggests that the endocytosis and release into the cell cytoplasm of RCA_{II} where it can directly interact with polyribosomes is the natural mode of action of this toxin. Other toxins such as diphtheria toxin may enter the cell to act in similar fashion[34,35].

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Serum dopamine β-hydroxylase activity in developing hypertensive rats

SPONTANEOUSLY hypertensive (SH) rats[1] have increased[2,3] tyrosine hydroxylase and dopamine β-hydroxylase (DBH) activities in the adrenal glands. They also show a pronounced hypotensive reaction[2,3] to the specific microbial inhibitors of these enzymes[4]. The results suggest some involvement of the adrenergic nervous system in the hypertension. But the activity of DBH in serum of SH rats, which is released from the sympathetically innervated organs and adrenals[5], does not differ significantly from that of normotensive Wistar rats[6].

We have now compared the developmental change in serum DBH activity in SH rats with that in normotensive Wistar–Kyoto rats. It was from rats of this strain that SH rats were bred, so we felt that they would be better controls than the previously used normotensive Wistar rats. We found that the serum DBH activity in young rats was higher in the SH than in the Wistar–Kyoto strain. We also examined the effect of NaCl administration on serum DBH activities. We found that in 10-week-old rats it caused the enzyme activity to increase simultaneously with a rapid increase in blood pressure, indicating activation of the sympathetic nerves.

SH rats and Wistar–Kyoto rats of the same age were raised in the same conditions. Blood pressure was measured in unanaesthetised animals using the indirect plethysmograph[1]. Blood samples were obtained from rats by decapitating and exsanguinating them into a test tube kept in ice, and serum was removed after centrifuging at 10,000*g* for 10 min. The enzyme activity was assayed by sensitive dual-wavelength spectrophotometry using tyramine as substrate[7]. This method permits the assay of the maximum velocity at a saturated substrate concentration (20 mM) under the optimum *p*H (5.0). The incubation mixture (total volume 1.00 ml) contained: 100 μl of serum diluted to 400 μl with water, 200 μl of 1 M sodium acetate buffer, *p*H 5.0, 100 μl of 0.1 M N-ethylmaleimide, 50 μl of 20 μM $CuSO_4$, 50 μl of 0.2 M sodium fumarate, 50 μl of 0.2 M ascorbic acid, 50 μl of 20 mM pargyline, 1,500 units of catalase in 50 μl of aqueous solution (1 mg ml^{-1}), and 50 μl of 0.4 M tyramine. A sample of boiled enzyme preparation was used as control. Incubation was carried out for 45 min at 37° C. The product, octopamine, was isolated by a microcolumn (0.5 × 10 cm) of Dowex-50-H^+ (packed volume, 0.2 ml), and converted by $NaIO_4$ (10μl of 2% aqueous solution) to *p*-hydroxybenzaldehyde which was then isolated by successive extractions with ether and ammonia, and finally measured by a dual-wavelength spectrophotometer at 333 nm and 360 nm. Octopamine (2.00 nmol) was added to a control incubation as internal standard. The activity was expressed as nmol octopamine formed per min per ml serum.

Table 1 shows that serum DBH activity of both SH and Wistar–Kyoto rats decreased markedly with age after 6 week. This contrasts with the situation in which humans DBH activity increases with age, especially after an erect posture is assumed[8]. The decrease in serum DBH activity could not result from formation of endogenous inhibitors in serum during development, since recovery of pure DBH from bovine adrenal glands added to a rat serum sample was similar (95%) from rats of various ages. One possibility is that the rate of development of the sympathetic nerves in rats may be slower than the increase of blood volume, so that the DBH activity in a volume of serum may decrease with age. The difference between humans and rats in this respect may have the same explanation as the observation that human serum contains a much higher enzyme activity than sera from various animals[9].

The enzyme activity in SH rats after 6 weeks of age is not significantly different from those in Wistar–Kyoto rats. This agrees with our previous result on serum DBH activities of SH rats and normotensive Wistar rats at 16 weeks of age[6]. But at 3 weeks of age, serum DBH activity of SH rats was about two times higher than that of Wistar–Kyoto rats, indicating that the sympathetic nerve activity in SH rats may be increased only when SH rats are very young. The loss of difference between serum DBH activities of SH rats and Wistar–Kyoto rats during development may

Table 1 Serum dopamine β-hydroxylase activities of developing spontaneously hypertensive (SH) rats

Age (weeks)	DBH activity (nmol min^{-1} ml^{-1} serum ± s.e.m.) Wistar–Kyoto rats	SH rats
3	1.18 ± 0.03 (5)	2.23 ± 0.09* (4)
6	0.44 ± 0.02 (4)	0.33 ± 0.01 (4)
12	—	0.22 ± 0.01 (4)
14	0.19 ± 0.04 (6)	0.19 ± 0.03 (6)
	—	0.30 ± 0.07† (10)
16	—	0.17 ± 0.03 (4)
53	0.20 ± 0.02 (4)	0.19 ± 0.03 (4)

*Differs from control (Wistar–Kyoto rats), $P<0.01$.
†NaCl was administered from 10 weeks of age for 4 weeks. Differs from control (SH rats without NaCl administration), $P<0.05$. Numbers in parentheses refer to no. of rats.

be due to a secondary compensatory mechanism in the course of the development of hypertension.

In order to determine the reactivity of the sympathetic nervous system of SH rats, we administered NaCl by supplying 1% NaCl-aqueous solution for drinking instead of tap water after 10 weeks of age for 4 weeks. The blood pressure of control SH rats without NaCl administration was increased from 170 ± 3 mm Hg (at 10 weeks) to 189 ± 3 mm Hg (at 14 weeks), whereas that of SH rats with NaCl administration increased from 173 ± 2 mm Hg (at 10 weeks) to 204 ± 5 mm Hg (at 14 weeks). Thus NaCl administration to SH rats increased blood pressure more pronouncedly. After NaCl administration serum DBH activities were also increased significantly as shown in Table 1. The results suggest that the sympathetic nerve activity in SH rats may be increased only when they are very young and decreased by a compensatory mechanism during the development of hypertension, but may be easily activated if blood pressure is raised acutely.

The elevated serum DBH levels when SH rats are very young and the rapid increase in serum DBH activity during the rapid increase of blood pressure by NaCl administration suggest some role for the sympathetic nervous system in the onset and maintenance of hypertension in SH rats.

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Enzymatic synthesis of acetylcholine by a serotonin-containing neurone from *Helix*

Acetylcholine (ACh) and serotonin are strong neurotransmitter candidates in the snail species *Helix aspersa* and *Helix pomatia*[1,2]. Experiments[1] have indicated that the enzymes responsible for their biosynthesis, choline acetyltransferase (ChAc, EC 2.3.1.6.) and L-aromatic amino acid decarboxylase (AAD, EC 4.1.1.26.), were specifically localised to separate populations of neurones in *H. aspersa*, and similarly for *H. pomatia* (M.R.H., unpublished). Out of 150 neurones, 36 were found to contain ChAc only (detection limit 2 pmol h^{-1}) and 20 were found to contain AAD only (detection limit 8 pmol h^{-1}). This clear biochemical differentiation between presumably 'cholinergic' neurones and presumably 'serotonergic' neurones has been previously noted in other invertebrates[3,4]. It was therefore surprising when, in two laboratories (St Andrews and Kjeller), we independently found that the two homologous giant metacerebral neurones, which contain serotonin[5] and are thought to use it as a neurotransmitter[6], could also enzymatically synthesise ACh. The coincidence of these two enzymatic activities in the giant serotonin cell (GSC) is a unique and therefore intriguing situation among characterised invertebrate nerve cells.

In experiments at Kjeller, ChAc was assayed as described by Fonnum[7] with modifications in the incubation medium to yield optimal activity for *Helix*[8]. In experiments at St Andrews, ChAc was assayed by the same method as modified by Coggeshall *et al.*[9] using the following incubation medium (final concentration): 200 μM ^{14}C-acetyl-CoA, 20 mM choline chloride, 300 mM NaCl, 1 mM EDTA. $2H_2O$, 80 mM sodium phosphate buffer (*p*H 7.4), 0.1 mM eserine, and 0.5% (v/v) Triton X-100. The cells were incubated for 1 h at 37° C (to give maximal *in vitro* activity), and the blanks were 1.5–2.0 pmol. In one series of experiments, both ChAc and AAD were assayed in the same incubation, using the following medium: 0.45 mM ^{3}H-acetyl-CoA, 0.2 mM D,L-1-^{14}C-DOPA, 8 mM choline bromide, 250 mM NaCl, 20 mM sodium phosphate

Table 1 ChAc activity of the giant metacerebral cells (GSCs) after different treatments

Treatment	Activity (pmol $cell^{-1}$ h^{-1}) *H. aspersa*	*H. pomatia*
Control	22.3±6.3(4)	20.6±2.0(8)
Incubation medium + AChE-eserine	<2 (3)	<2 (3)
Incubation medium—choline	<2 (3)	<2 (3)
Incubation medium + Acryloylcholine (1.25 mM)	<2 (3)	<2 (5)
Incubation medium + Chloroacetylcholine (0.125 mM)	<2 (3)	<2 (5)
Incubation medium + CoA (100 mM)	10.8±3.7(3)	10.4±2.2(3)
Incubation medium + ACh (20 mM)	13.0±2.9(3)	11.4±2.5(3)
Incubation medium + $MgCl_2$ (100 mM)	8.9±4.2(3)	11.2±2.6(3)
Incubation medium + $CaCl_2$ (100 mM)	3.6±1.7(3)	5.5±2.3(3)
Heat denaturation (1 h at 90° C)	<2 (3)	<2 (7)
TCA precipitation	<2 (3)	<2 (7)

Results are expressed as means ± s.e.m. The numbers in parentheses refer to no. of values used for mean.

buffer (*p*H 7.4), 0.1 mM eserine, 0.1 [illegible] M pyridoxal phosphate, 0.125 mM nialamide, 11.5 mM EDTA, and 0.2% (v/v) Triton X-100. The assay was based on the absorption in hyamine of the evolved ^{14}C-CO_2 from DOPA, and the extraction of ^{3}H-ACh by Kalignost as before[7]. Animals were maintained in artificial hibernation by storage in a humid environment at 10° C. The dissection was performed as described elsewhere[1]. In some experiments, the removal of single cells was expedited by conducting the dissection under 70% ethylene glycol (−20° C). Enzyme activities were found to be unaffected by the choice of dissection solution, as noted earlier[4]. All experiments were conducted in late spring and summer.

The identification of the extractable radioactivity as acetylcholine was based on the following observations (see Table 1). First, electrophoresis of the extract gave a single radioactive product comigrating with standard ACh (Fig. 1) in two sets of conditions[4,10]; second, no activity was found in the absence of choline; third, no activity was found in the presence of acetylcholinesterase and the absence of cholinesterase inhibitor and, fourth, Kalignost extraction completely separated ACh from acetylcarnitine and other possible acetylated contaminants[11].

Synthesis of ACh was abolished on boiling the cells or after trichloroacetic acid (TCA) precipitation (Table 1), indicating that it is enzymatically mediated. The enzymatic ACh synthesis was inhibited by the presence of $MgCl_2$, $CaCl_2$, ACh, CoA, acryloylcholine, and chloroacetylcholine, in concentrations known to similarly affect the partially purified ChAc from whole snail brain[8]. The latter two substances should be regarded as fairly specific ChAc inhibitors, and inhibited the GSC enzyme to the same degree as they did other choline acetyltransferase-containing neurones.

The level of ACh synthesis by the GSC was highly reproducible in this assay. It was lower than that of a neurone found on the posterio-dorsal surface of the visceral ganglion (known as cell no. 21 (ref. 1) on cell no. 4 (ref. 12)), but comparable to that of the medial buccal cell (cell no. 4 (ref. 1)), which was considered to have a relatively low ChAc activity. Other cells known to contain large amounts of serotonin or AAD, such as the one localised near the right parietal ganglion in the posterio-dorsal surface of the visceral ganglion (known as cell no. 11 (ref. 1) on cell no. 4 (ref. 13)), contained insignificant ChAc activity. These cells served as controls that the observed ACh synthesis in the GSC was not in any way associated with serotonin-metabolising enzymes. Using the double assay, the uniqueness of the GSC was reaffirmed as both enzymes could indeed be measured simultaneously, whereas in control cells, either one activity or none was observed (Table 2). A control extract of 0.5 µl dissecting medium taken from the area of the GSC dissection did not reveal any background activity liberated by tissue disruption (Table 2).

Examination of related species having GSCs[5] revealed that they also share this dual-enzyme characteristic in these neurones, albeit the activity varies somewhat (M.R.H., G.A.C., P.C.E., and F.F., unpublished).

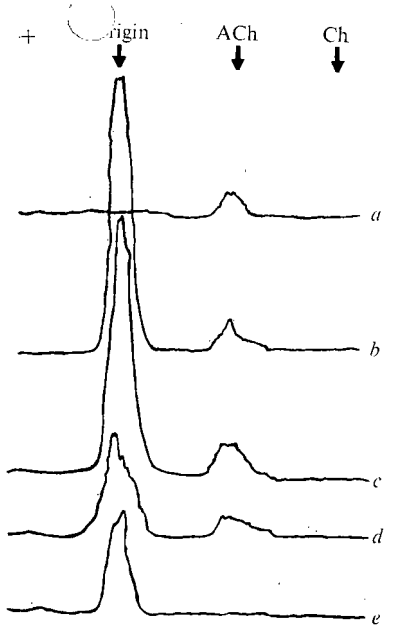

Fig. 1 Electrophoresis of the choline acetyltransferase assay product. Samples were electrophoresed for 50 min in the conditions of Potter and Murphy[9], and subsequently scanned for radioactivity in a Nuclear Chicago Actigraph III (960 V high voltage, 1 s counting, chart speed of 240 cm h^{-1}, 6 mm collimator slit width). Scans *a* and *b* represent the results of assays using a 0.8 µl extract of snail brain homogenate as the source of enzyme. In *a*, 50 µl of Kalignost extraction of the completed incubation was applied. In *b*, 2 µl of the unextracted, completed incubation was applied. In *c*, six GSCs were used as the enzyme source, and in *d*, one cell 21 neurone. Scan *e* is the control to which no enzyme was added. Acetylcholine (ACh) and choline (Ch) mobilities were determined by staining added standards with iodine vapour.

There is consequently strong evidence that acetylcholine can be synthesised in a known serotonin-containing neurone by a choline acetyltransferase.

Assuming that the ChAc serves a physiological role of ACh production in the GSC, it is worthwhile considering the functions of ACh and serotonin within the neurone. Both may serve as neurotransmitters, in which case the long-held dogma of each neurone making and utilising a single neurotransmitter must be abandoned[14]. In this regard, it should be noted that the GSC ChAc is more active than the base levels we quote here during those months when it is difficult to elicit responses to serotonin from the follower cells[6]. Far more likely is the possibility that either the ACh or serotonin (or both) serves as alternative cellular or hormonal function besides classical neurotransmission, perhaps modulating metabolic activity of innervated regions. Last, it should be considered that the ChAc activity might arise from contamination, either by invaginating glia or adhering microneurones. This can never be excluded when work-

Table 2 Enzyme activities of single neurones using both single and double assays for ChAc and AAD in *Helix aspersa*

Neurone	Location	Double assay		
		ChAc activity pmol $cell^{-1}$ h^{-1}	ChAc activity pmol $cell^{-1}$ h^{-1}	AAD activity
GSC	Ventral surface of metacerebral ganglion, near input of inner lip nerve	22.3±6.3(4)*	19.8±9.0(6)	26.0±14.3(6)
Medial buccal cell	Lateral edge of dorsal surface of buccal ganglia. Medial of group of three giant neurones	21.0±5.0(4)	20.3±10.2(3)	<8 (3)
Cell 21	Posterio-dorsal surface of visceral ganglion, between inputs of cutaneous pallial nerve and visceral nerve	65.6±7.9(5)	56.3±12.0(3)	<8 (3)
Cell 11	Posterio-dorsal surface of visceral ganglion, bordering right parietal ganglion	<2 (5)	<2 (3)	56.4±7.5(3)
Posterior buccal cell	As medial buccal cell. Posterior of group of three giant neurones	<2 (5)	<2 (3)	<8 (3)
	Control extract from area of GSC dissection (0.5 µl)	<2 (8)		

* Numbers in parentheses refer to number of determinations used to derive figure.

ing with hand-dissected preparations[15]; but the specific localisation of the enzymes to particular neurones and the absence of ChAc activity from the dissection area do not support this objection.

The possibility that the ACh in the conditions of the assay, is synthesised by an enzyme other than ChAc cannot be excluded. It seems very unlikely, however, in view of the effect of the ChAc inhibitors. Carnitine acetyltransferase (EC 2.3.1.7.) and aryl amine N-acetyltransferase (EC 2.3.1.5.) have been discounted on the basis of their hundredfold lower activities and different kinetic parameters (P.C.E., and M.R.H., unpublished).

Further work will focus on the direct measure of ACh and its functional role in the GSC.

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Assignment of the gene for galactokinase to human chromosome 17 and its regional localisation to band q21-22

PROGRESS in somatic cell genetics now allows more rapid and precise localisation of genes within the human genome. A large number of mouse × human hybrid cell lines are available, permitting the investigator to choose, for phenotype assay, lines with a particular reduced human chromosome complement. An increasingly large number of hybrid lines carrying rearranged chromosomes are also becoming available for regional localisation studies. We have used such hybrid cell lines to assign a gene coding for galactokinase (EC 2.7.1.6) to human chromosome 17 and to further localise the gene to band 21–22 on the long arm of the chromosome. Results from this study have provided new information regarding the functioning of the genes coding for the Leloir pathway enzymes in man[1], and have revealed a relatively close and potentially useful linkage between the genes for galactokinase and for thymidine kinase (EC 2.7.1.21).

The 70 mouse × human cell lines used in this analysis were produced, selected and propagated by methods reviewed elsewhere[2]. Hybrid series 41 is composed of 14 primary cell lines, 11 of known independent origin, isolated after fusion of mouse temperature sensitive line ts-1 (ref. 3) with GM 126, human skin fibroblasts. Series 50c consists of 5 primary lines, 4 of known independent origin, isolated after fusion of mouse line TA3 (ref. 4) with human peripheral blood leukocytes. The AIM series consists of 11 primary lines, 9 of known independent origin, isolated after fusion of mouse line A9 (ref. 5) with human skin fibroblasts. The YA series consists of 20 primary lines, 8 of known independent origin, isolated after fusion of A9 with human skin fibroblasts.

We also used a panel of unrelated hybrid lines chosen from a collection of mouse × human hybrids on the basis of their specific partial human karyotypes. Only a few different human chromosomes are represented within a given hybrid line, the same chromosomes being present in many or most of the individual cells. The specific human chromosomes represented varied widely between hybrids of the panel. Analysis of this set of cell lines, then, generally allows the rapid and unequivocal correlation of a given human phenotype with a particular human chromosome. Hybrids which composed the panel were five subclones of JFA-14a and six of JFA-16a (two independent hybrid lines resulting from a fusion of A9 with human skin fibroblasts[6]), WA-Ia-D6[6] (A9 × WI-38[7], human embryonic lung fibroblasts) and IL-II-5[8] (mouse L cell line LM(TK$^-$)[9] × IMR 32, human neuroblastoma).

For regional localisation studies we used two hybrid lines from the WL series[10], WL-24a-2-A and WL-24a-5-C, plus five subclones of WL-24a-2-A isolated after treatment of the hybrid line with adenovirus-12 (ref. 11). WL hybrids originated from a fusion of LM(TK$^-$) with WI-38. They were maintained in HAT selective medium[12] in which cells retaining the human thymidine kinase gene complementing the mouse deficiency survive. Neither of these two hybrids, however, retained an intact human chromosome 17, the chromosome to which the cytosol form of thymidine kinase has been assigned[13–15]. In WL-24a-5-C a portion of the long arm of 17 persists as a marker chromosome, t(11; 17) (q22; q21), which arose

Table 1 Enzymatic activities assayed in hybrids and their respective human chromosome assignments

Chromosome		Enzyme
1	Pep C	peptidase C (EC 3.4.3.–)
2	IDH-1	isocitrate dehydrogenase (EC 1.1.1.42)
2	MDH-1	(NAD)-malate dehydrogenase (EC 1.1.1.37)
6	ME-1	(NADP)-malic enzyme (EC 1.1.1.40)
7	MPI	mannose phosphate isomerase (EC 5.3.1.8)
10	GOT	glutamate oxaloacetate transaminase (EC 2.6.1.1)
11	LDH A	lactate dehydrogenase (EC 1.1.1.27)
12	Pep B	peptidase B (EC 3.4.3.–)
14	NP	purine nucleoside phosphorylase (EC 2.4.2.1)
16	APRT	adenine phosphoribosyltransferase (EC 2.4.2.7)
18	Pep A	peptidase A (EC 3.4.3.–)
19	GPI	glucose phosphate isomerase (EC 5.3.1.9)
20	ADA	adenosine deaminase (EC 3.5.4.2)
21	SOD-1	cytoplasmic indophenol oxidase or superoxide dismutase-1
X	G6PD	glucose-6-phosphate dehydrogenase (EC 1.1.1.49)
X	PGK	phosphoglycerate kinase (EC 2.7.2.3)

Table 2 Number of hybrids showing presence (+) or absence (−) of the two given human enzymatic activities

	+/+	+/−	−/+	−/−	Total
GaK/IDH-1	5	18	3	24	50
GaK/MDH-1	6	17	7	20	50
GaK/Pep A	17	6	10	17	50
GaK/TK	16	2	0	20	38

Hybrids assayed for IDH, MDH-1 and Pep A are from series 41, 50c, AIM and YA. Hybrids assayed for TK are from series AIM, YA and WL.

spontaneously in the hybrid[16]. In WL-24a-2-A the entire long arm of 17 has been translocated to a mouse chromosome, (M; 17q). In both cases, the translocated product represents the sole identifiable human chromosome element in the hybrid. Treatment of WL-24a-2-A with adenovirus-12 induced breaks in the translocation chromosome (M; 17q) specifically in the region 17q21-22. In subclones A12/1B and A12/1D, the break occurred in 17q21. In subclones A12/2A, A12/2C, and A12/2D, a break occurred in 17q22. Terminal deletions occurred distal to these breaks. These lines have been used previously to assign the gene coding for thymidine kinase to human chromosome band 17q21-22 (ref. 11).

Extracts of the hybrid lines were assayed by vertical starch gel electrophoresis for the presence of human electrophoretic forms of 16 enzymes. Genes coding for these enzymes have previously been mapped to specific human chromosomes, thus the presence of the human isozyme is considered to be strong evidence for the presence of the corresponding human chromosome in the hybrid line. The enzymes used as markers in this study are listed in Table 1 with their chromosome assignments. Methods of assay have been described elsewhere[17]. Thymidine kinase activity was assayed on Cellogel (Chemetron, Milan, Italy) using the method of Migeon *et al.*[18]. Electrophoresis was carried out at 4° C for 4 h at 30 V cm^{-1} using a buffer of 50mM TES (N-*tris*(hydroxy-methyl)methyl-2-aminoethane sulphonic acid) adjusted to *p*H 6.2 with triethanolamine. Our method used to assay galactokinase activity on vertical starch gels has been described[19], (Fig. 1).

Enzymatic analysis of the hybrids from series 41, 50c, AIM and YA indicated that galactokinase (GaK) activity segregated discordinately with the 16 enzymes used as chromosome markers. A representative data sample given in Table 2 shows discordant segregation of GaK with IDH-1 and MDH-1, two markers on chromosome 2 and with Pep A, a marker for chromosome 18. These two chromosomes plus 12 others corresponding to the remaining 13 enzymes could thus be eliminated from consideration in attempts to assign the gene coding for GaK activity to a particular human chromosome. When the panel of well-karyotyped hybrid lines were assayed or GaK activity (Table 3) and conversely the 11 AIM hybrids were karyotyped this conclusion was further substantiated. The 14 chromosomes which were eliminated from consideration on the basis of the enzyme assays could also be eliminated on the basis of chromosome analysis of the hybrids. Nine additional chromosomes which either carried no easily assayed enzyme marker or carried markers which were not included in our assays could also be eliminated from consideration on the basis of chromosome analysis. It then became clear that only chromosome 17 segregated concordantly without exception with GaK activity. The gene for GaK was assigned on this basis to human chromosome 17. The assignment was reinforced by thymidine kinase (TK) assay of the AIM, YA, and WL hybrids. With only two exceptions where human GaK appeared to be present in a hybrid and TK not, the two enzymatic activities segregated concordantly (Table 2).

More precise localisation of the GaK gene along the length of chromosome 17 was made possible by analysis of the WL hybrids. Cell line WL-24a-2-A carrying the (M; 17q) translocation chromosome retained human GaK activity restricting the map position of the gene to the long arm of the chromosome. Hybrid WL-24a-5-C which carried the t(11; 17) (q22; q21) marker likewise showed human GaK activity further restricting the gene to a position in or distal to 17q21. WL-24a-2-A subclones A12/1B and A12/1D which had lost the chromosome portion distal to a break in 17q21 had also lost GaK activity. Subclones A12/2A, A12/2C and A12/2D in which the chromosome loss involved only regions distal to the break in 17q22 retained GaK activity. The GaK gene, from this evidence, must be located between the two breakpoints, and we have accordingly assigned the gene to human chromosome 17 band q21-22 (Fig. 2).

Analysis, then, of the chromosome and enzyme complement of a large number of independent hybrid cell lines of diverse mouse × human parentage has yielded compelling evidence for the assignment of the galactokinase gene to human chromosome 17. GaK activity segregated discordantly with 16 marker enzymes and concordantly with only chromosome 17 and TK activity. Data from two WL subclones which were functionally back-selected for loss of TK activity indicate that GaK activity is lost concordantly with the loss of a portion of chromosome 17. Information obtained from the WL hybrids allowed us to further restrict the map position of the galactokinase gene to band q21-22. The discrepant data which we have obtained, that regarding discordant segregation of TK with GaK in two YA hybrids, could be explained either by the difficulty in assaying TK or by a possible breakage and rearrangement of chromosome 17. We have not been able to identify an intact 17 in one of the discrepant hybrids.

Only chromosome 17 is consistently present in hybrid lines which show human GaK activity as substantiated by the chromosome data (Table 3) and the complementary results

Table 3 Human GaK activity and human chromosomes present in hybrid cell lines

Hybrid	Human GAK	Human chromosomes 1	2	3	4	5	6	7	8	9	10	11	12	X	13	*14	15	16	17	18	19	20	21	*22	Y
JFA-16a-5	−		+			+			+			+				+				+		+		+	
JFA-16a-8	+		+			+	+		+			+				+		+		+		+		+	
JFA-16a-15b	−		+			+						+							+	+		+		+	
JFA-16a-19	−		+			+						+								+		+			
JFA-16a-21	−		+									+				+				+		+		+	
JFA-14a-5	+											+								+		+			
JFA-14a-10c	+	+	+			+			+						+			+	+	+					
JFA-14a-12	+		+												+				+	+					
JFA-14a-13	+		+			+												+	+	+					
JFA-14a-23b	+		+															+	+	+					
JFA-14a-6b	+		+															+	+	+					
WA-Ia-D6	−	+	+		+					+	+	+	+	+	+			+	+	+		+	+		
IL-II-5	+								+				+						+		+				
*t(14;22).																									

Karyotypes of hybrids were analysed using air dried preparations[24] stained with quinacrine mustard or Atebrin to produce fluorescent banding of the chromosomes[25] and stained subsequently with Giemsa to reveal constitutive heterochromatin[26]. At least 20 cells from each hybrid line were analysed for the presence of human chromosomes. In all cases specific chromosomes were scored as present in the hybrid if they were found in at least 10% of the cells examined.

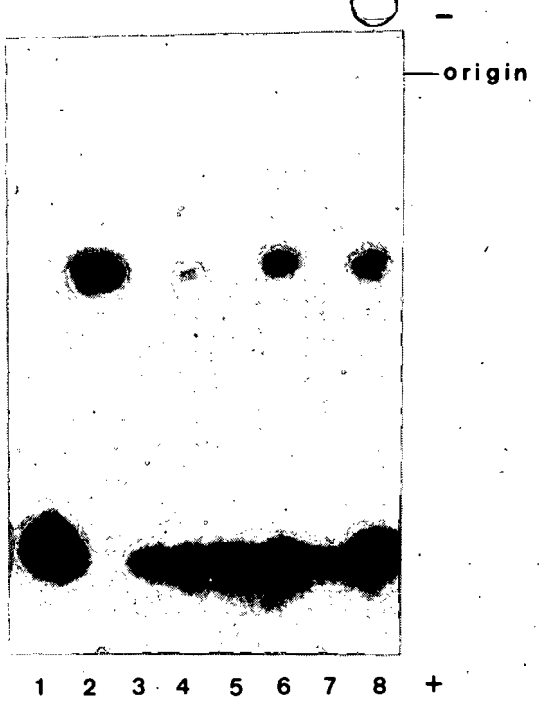

Fig. 1 Galactokinase assay of five WL-24a-2-A subclones. After electrophoresis in citrate phosphate buffer at *p*H 6.8, the gel was incubated in the reaction mixture of Mayes and Guthrie[27]. ^{14}C-galactose-1-phosphate produced by the enzyme was then precipitated in the gel with lanthanum chloride and subsequently detected by autoradiography[28]. Omission of ATP from the reaction mixture prevented the appearance of the reaction spots. Addition of 10 mM D-glucose did not. Mouse control, LM(TK$^-$), was run in channel 1. Human control, KB, is shown in channel 2. Channels 4, 6 and 8 represent extracts of subclones A12/2C, A12/2A and A12/2D, respectively, all of which show both mouse and human forms of the enzyme. Channels 3 and 7 represent extracts of A12/1B and channel 5 represents A12/1D, neither of which shows the human activity band.

from enzyme analysis (Table 2). In addition, hybrid WL-24a-2-A which shows human GaK activity carries the long arm of 17 as its sole human chromosomal element. For this reason we feel that the genetic locus which we have mapped codes for a structural element of the enzyme. In this respect it might be noted that galactokinase purified from human red blood cells is reported to consist of two subunits of approximately equal size with only the dimeric form showing enzymatic activity[20]. We find only one spot of activity for human galactokinase by our assay method and might suppose that it represents this dimer. It is surprising then that no heteropolymer of intermediate electrophoretic mobility forms between human and mouse isozymes in the hybrids as has been observed with numerous other multimeric enzymes[17].

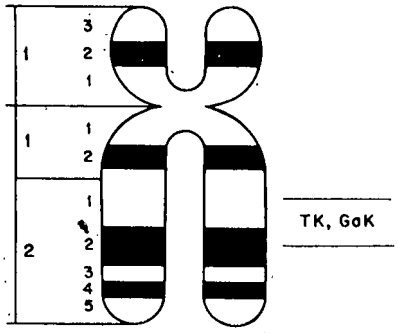

Fig. 2 Chromosome 17 as given by the Paris Conference (1971) (ref. 29) indicating the assigned map position for GaK.

Galactokinase is the first of three enzymes of the Leloir pathway. Since the structural genes for these enzymes map within the same operon in *Escherichia coli*[21] and are closely linked also in yeast, *Saccharomyces cerevisiae*[22], it is interesting to know whether the corresponding genes are linked in man. Human galactose-1-phosphate uridyltransferase (EC 2.7.7.12) has been assigned in two conflicting reports to chromosome 2 (ref. 23) and 3 (personal communication from T. A. Tedesco), and we assign galactokinase to 17. It seems, then, that the genes coding for these two enzymes of the pathway in man are probably not linked.

Linkage of the gene coding for galactokinase with that for thymidine kinase on the other hand, is one of the closest linkages to be detected using the techniques of somatic cell genetics. Since thymidine kinase is a widely used selective marker, these two genes form an especially useful gene pair. For example, it will be of interest to test the possible coordinate transfer of these markers in gene transfer experiments.

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5-Methylcytosine localised in mammalian constitutive heterochromatin

DEOXY-5-METHYLCYTIDYLIC acid (5-MeC) is an almost universal, although minor component of DNA in plants and animals. It makes up 4–7% of the bases in plants[1], no more than 1.5% of the bases in the mouse[2] and even less in the human[3]. 5-MeC itself is not incorporated directly into DNA. Instead, specific deoxycytidylic acid residues in preformed DNA are methylated enzymatically by a highly specific DNA methylase, which transfers a methyl group from S-adenosyl methionine to specific sites in DNA[4]. In the mouse, as in other mammals, virtually all of the 5-MeC is in the 5-MeCpG doublet[5], which is distributed non-randomly in the DNA. Mouse satellite DNA contains about 3% 5-MeC in comparison to about 1.3% in main band DNA[2] and is located at the centromeric end of nearly every chromosome[6].

Antisera to 5-methylcytosine (anti-5-MeC) are available (ref. 7 and Sawicki, Erlanger and Beiser, unpublished) and these can react with 5-MeC residues in single-stranded DNA. Earlier we showed that antibodies specific for particular purine or pyrimidine bases[8], which react with denatured DNA but not native DNA[9], can bind to fixed metaphase chromosomes. The major sites of binding can be localised by immunofluorescence[10,11]. These antibodies can therefore serve as probes of mammalian chromosome organisation. After ultraviolet irradiation, for example, of fixed mouse or human chromosomes, anti-adenosine (anti-A) and anti-thymidine antibodies bound intensely to the AT-rich constitutive heterochromatin[12].

We have now investigated the chromosomal location of 5-MeC residues in both the mouse and the human. Chromosome preparations which had been either ultraviolet irradiated or heated to 85° C in 0.07 M Sorensen's buffer at *p*H 6.5 for 15 or 30 min and chilled suddenly to prevent reassociation, were exposed to one or other of two different anti-5-MeC antisera. These antisera showed no cross reaction with thymidine or with cytidine after absorption with an excess of cytidine conjugated to rabbit serum albumin. The immunofluorescent banding of chromosomes with these two antisera was thus a result of specific binding to 5-MeC residues in the DNA and could be totally abolished by competition with added 5-MeC.

In the mouse, anti-5-MeC showed preferential binding to the C-band regions of centromeric heterochromatin (Fig. 1). The fluorescent regions were very small on a few of the larger chromosomes. These appeared to be the same chromosomes (1 and 3) which have very little centromeric heterochromatin in the AKR strain of mouse used in these experiments[13]. The intense fluorescence of the C-band regions partly reflects the greater concentration of 5-MeC in mouse satellite DNA than in main band DNA[2], but also the greater degree of ultraviolet-induced strand separation in the very AT-rich satellite DNA, which contains sequences of up to four thymine residues in a row in its light strand, and up to five in its heavy strand[14].

The presence of 5-MeC in mouse satellite DNA indicates that the repeating nucleotide sequence in this DNA contains the 5-MeCpG doublet. The amount of 5-MeC, 3 mol% (ref. 2), sets a lower limit on the length of the repeating sequence in the satellite DNA. Assuming that each strand is methylated equally, and taking into account the presence of a small proportion of non-identical sequences, this repeating sequence must be about 30 nucleotides long or some multiple of this. This fits rather well with the estimates of Hutton and Wetmur[15] and Sutton and McCallum[16]. Sutton's estimate of 8–13 nucleotides[17] is clearly too short unless the repeating sequence contains even shorter repeating sequences, some of them not containing 5-methylcytosine.

In the human, the pattern of anti-5-MeC binding was virtually identical whether strand separation was achieved by ultraviolet irradiation or by heating to 85° C. In both cases anti-5-MeC showed preferential binding to the C-band regions of constitutive heterochromatin of chromosomes 1, 9 and 16, the centromere–short arm region of chromosomes 15, and the distal part of the Y (Fig. 2). The C-band region of chromosomes 10, 17, 22 and Y sometimes showed a small region of enhanced anti-5-MeC binding. The observed pattern of binding, and the absence of comparable binding of anti-5-MeC to the C-band regions of most of the chromosomes suggests that methylation occurs only in certain clases of repetitive DNA. For example, the strongly positive regions in chromosomes 1 and 16 are the chief sites

Fig. 1 Metaphase spread from an AKR strain mouse showing sites of anti-5-MeC binding. Note the bright fluorescence at the centromeric end of most chromosomes and the low level of fluorescence of the remainder of all the chromosomes. Mitotic cells from a primary embryonic culture were expanded with 0.075 M KCl hypotonic solution, fixed in three changes of methanol:glacial acetic acid (3:1) and air dried. The spreads were ultraviolet irradiated for 16 h (30 cm from a germicidal lamp) either dry or in Sorenson's buffer, incubated for 30 min with anti-5-MeC at room temperature, washed thoroughly with phosphate buffered saline (PBS: 20 g NaCl, 85 ml 0.25 M Na_2HPO_4, 15 ml 0.25 M KH_2PO_4 in 2,400 ml distilled water H_2O, *p*H 7.2–7.4), incubated for 30 min with fluorescein-tagged anti-rabbit gammaglobulin prepared in sheep, washed thoroughly with PBS, mounted in PBS and analysed by fluorescence microscopy using transmitted light from an HBO 200 W high pressure mercury lamp delivered through a cardioid condenser, BG-12 exciter filter and K530 barrier filter. The photograph was taken on Kodak Plus X film with a 2 min exposure, developed with Acufine and printed on Ilford No. 4 grade paper.

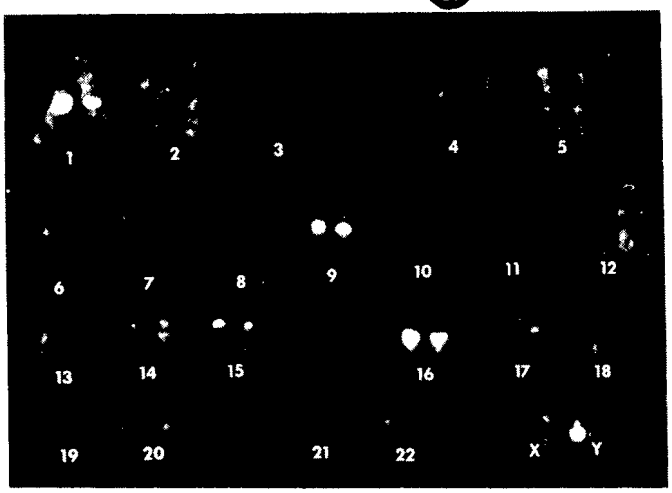

Fig. 2 Karyotype of a human cell showing sites of anti-5-MeC binding. Note the intensely bright fluorescent regions on chromosomes 1, 9, 15, 16 and the Y, and the difference between homologues in each case in the size of this region. Smaller bright centromeric dots are present on one or both copies of chromosomes 14, 17, 22 and Y. Technique as in Fig. 1. Chromosomes were identified by restaining the cell with quinacrine mustard and rephotographing, using the same fluorescence microscopy system and film but with an exposure of only 9 s instead of the 2 min necessary with the antibody.

of human satellite II DNA as revealed by in situ DNA–RNA hybridisation[18], whereas the corresponding 5-MeC-positive region of chromosome 9 contains both satellite II DNA[18] and satellite III DNA[19]. Our results suggest that satellite II, and perhaps satellite III, contain 5-MeC. We are unaware of a direct chemical analysis of the 5-MeC content of the various human satellite DNAs. Since neither satellite II nor satellite III is abundant in chromosome 15 or the Y, these chromosomes must contain different types of highly repetitious, methylated DNA.

The immunofluorescence approach described here can be used to locate classes of highly repetitive DNA which contain 5-MeC. It is already apparent that these classes show remarkable quantitative variation between homologues and among individuals. These differences provide chromosome markers which can be used in genetic linkage studies or in the analysis of structurally altered chromosomes. This might be particularly useful in identifying a structurally altered chromosome 15, since its proximal portion can now be more specifically identified than before. Striking differences have been noted in comparing anti-5-MeC binding to chromosomes of gorilla, chimpanzee and orang utan (unpublished data); and the technique seems to offer a promising approach to the evolution of satellite or other classes of highly repetitive DNA.

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Structure of DNA in DNA replication mutants of yeast

As in higher eukaryotes[1,2], the nuclear DNA of the simple eukaryote *Saccharomyces cerevisiae* is replicated from multiple internal initiation sites along the chromosome[3–5]. The very small size of the chromosomal DNA in yeast[3,6,7] and the availability of temperature-sensitive DNA synthesis mutants[8,9] make a detailed study of DNA replication easier in yeast than in most other eukaryotic systems. In this report, nuclear DNA molecules from several DNA synthesis mutants which had been classified as DNA initiation or DNA propagation mutants on the basis of incorporation kinetics were examined with the electron microscope. Our results provide independent criteria for the classification of DNA initiation and propagation mutants on the basis of the structure of the nuclear DNA at the restrictive temperature.

Three temperature-sensitive DNA replication mutants were used in these studies: *cdc*4, *cdc*7 and *cdc*8 (refs 8,9). If synchronous cultures of cells carrying mutations in *cdc*4 or *cdc*7 are shifted to the restrictive temperature immediately after initiation of DNA synthesis has occurred, approximately a complete round of DNA is made before synthesis stops[8,9]. It is been suggested, therefore, that the gene functions *cdc*4 and *cdc*7 are required for the initiation of nuclear DNA synthesis[8,9]. The gene function *cdc*8 is necessary for DNA propagation since cells carrying this mutation abruptly shut off nuclear DNA synthesis when shifted to the restrictive temperature[8].

When DNA molecules from *cdc*4 at the restrictive temperature were examined in the electron microscope, all molecules (126/126) were simple linear structures, containing neither replication 'bubbles' ('eye-forms') nor Y-shaped replication forks. In DNA isolated from *cdc*7 at the restrictive temperature (36° C), 144/150 molecules were simple linear structures. Six of the molecules contained replication forks, possibly the result of some leakiness of DNA syn-

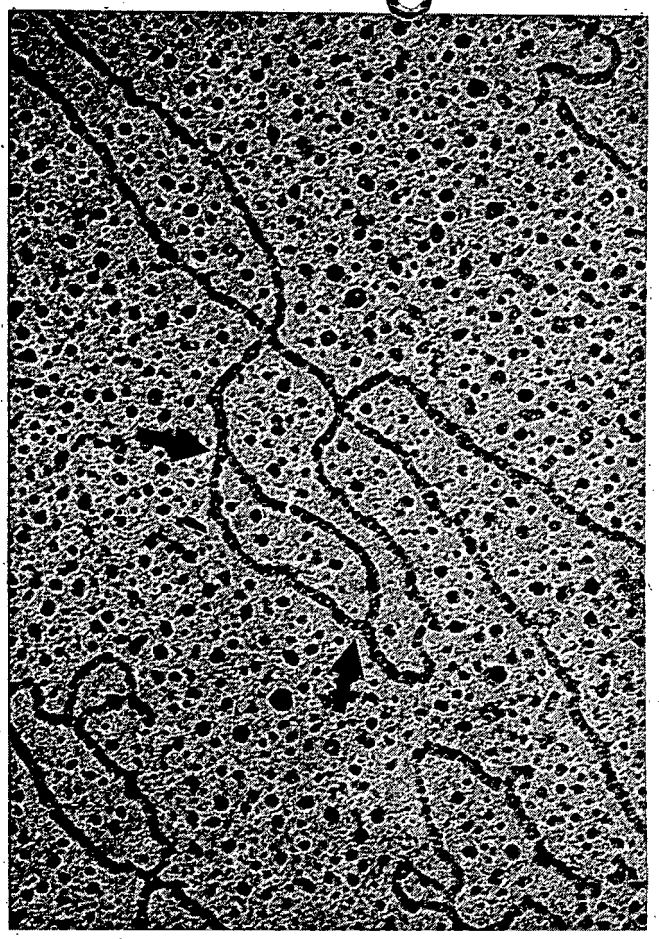

Fig. 1 Small DNA replication bubble in DNA isolated from a temperature-sensitive DNA propagation mutant (*cdc*8) at the restrictive temperature. A culture of *cdc*8 (strain 198–1) was grown at 23° C in 10 ml of Y-minimal media[6] supplemented with 0.2 g glucose, 5 mg yeast extract, 200 μg uracil, 200 μg adenine, 500 μg tyrosine, 500 μg histidine and 500 μg lysine. The doubling time of the culture under these conditions is approximately 3 h. At a cell density of 2×10^6 cells ml^{-1}, the culture was shifted to 36° C for 5 h. Cells were then collected and spheroplasted as described previously[6]. 0.2 M hydroxyurea (Matheson, Coleman and Bell, Norwood, Ohio) was added to the spheroplasting solutions. Nuclear DNA was separated from other cellular components by centrifugation in sucrose gradients[3]. Methods for spreading DNA for electron microscopy have been described[3]. Each branch of the bubble in the photographed molecule is 0.4 μm in length; arrows show the forks. Nuclear DNA was prepared from *cdc*4 (strain 135.1.1.) and *cdc*7 (strain 4008) as described above. For both initiation mutants all the DNA molecules observed in the experiments should come from cells arrested in the cell cycle at the beginning of the S period[8,9]. For *cdc*8, however, although most of the molecules of DNA should come from cells blocked near the beginning of the S period, some DNA molecules should come from cells within the S period. Since only 3–5% of the DNA molecules in an asynchronous culture are replicating at any given time[3], most of the DNA molecules observed in the *cdc*8 experiment should contain no replication structures before the shift to the restrictive temperature.

thesis at the restrictive temperature. This allele of *cdc*7 (4008) is somewhat leaky at 36° (ref. 9) synthesising nuclear DNA at approximately 10% the normal rate (C.S.N., unpublished observations). When the experiment is done at 38° C with *cdc*7, 149/150 DNA molecules were simple linear structures[4,5].

Hartwell[10] has pointed out the difficulties in distinguishing true DNA initiation mutants from mutants which have a leaky replication block, a replication block with a delayed onset of expression or a replication block early in the process of DNA replication. The observation that very few of the DNA molecules observed in DNA isolated from the initiation mutants *cdc*4 and *cdc*7 contain DNA replication intermediates argues that the cells are blocked before the onset of DNA replication. These data and the incorporation data from previous studies[8,9] indicate that *cdc*4 and *cdc*7 may be true DNA initiation mutants.

The findings with *cdc*8, the DNA propagation mutant, were quite different. Approximately half (114/240) of the DNA molecules isolated from *cdc*8 at the restrictive temperature contained one or more replication bubbles. Often these bubbles were very small, less than 2 μm in size (Fig. 1). Figure 2 is a histogram of bubble sizes. The average bubble (3 μm) observed in this experiment is less than that found in replicating molecules isolated from a synchronous early S-phase population of cells[4,5]. Figure 3 is a histogram of centre-to-centre distances between bubbles in molecules which contain more than one bubble.

The high proporation of DNA molecules with replication bubbles found in experiments with *cdc*8 indicate that this mutant can initiate DNA synthesis at the restrictive temperature. The spacings between adjacent bubbles (Fig. 3) are similar to those observed during normal DNA replication[4,5] suggesting that DNA synthesis may be initiating in *cdc*8 at the restrictive temperature at the same sites as during normal DNA replication. The small size of the replication bubbles probably reflects a much reduced rate of chain propagation at the restrictive temperature. The rate of nuclear DNA synthesis in *cdc*8 at the restrictive temperature is less than 1% the normal rate (C.S.N., unpublished observations). An alternative explanation for the small replication bubbles is that the *cdc*8 gene function is not required for replication of a small amount of DNA at the initiation site but is required for subsequent chain propagation. This explanation seems somewhat unlikely but cannot yet be ruled out. The small proportion of molecules with large replication bubbles may be molecules which contained replication intermediates at the time of the shift to the restrictive temperature (as discussed in the legend to Fig. 1). Therefore, the large number of DNA molecules in *cdc*8 at the restrictive temperature which contain small replication bubbles confirms the classification of *cdc*8 as a DNA propagation mutant. Hartwell[9] has recently classified another cell cycle mutant, *cdc*21, as a possible DNA propagation mutant on the basis of incorporation kinetics. Preliminary

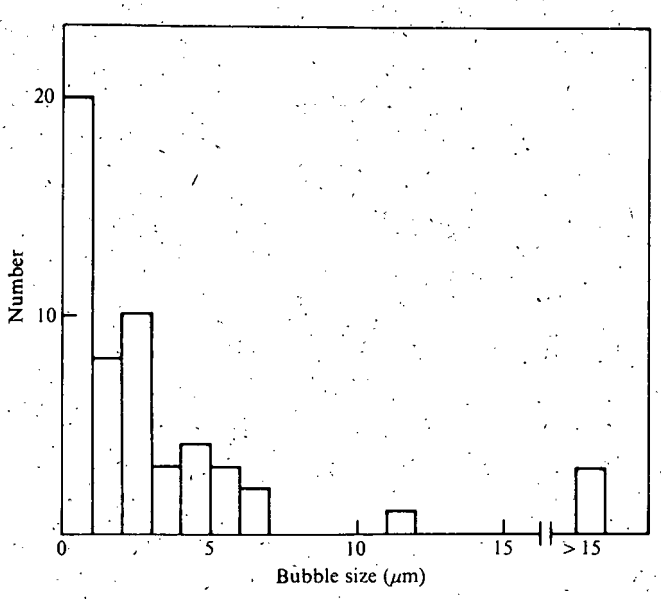

Fig. 2 Histogram of bubble sizes from DNA molecules isolated from *cdc*8 at the restrictive temperature. Photographs were printed at a final magnification of 10,000 to 25,000 and contour measurements were made with a Keuffel and Esser map measurer.

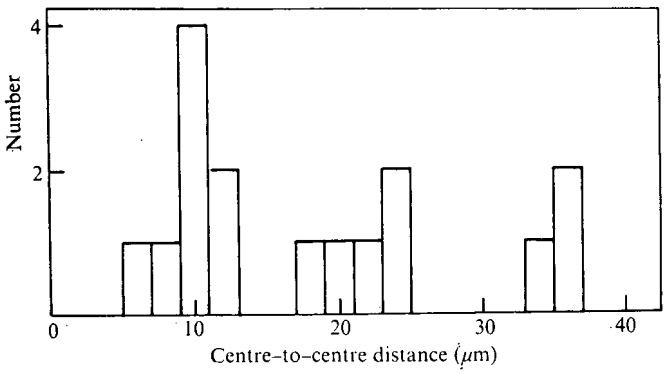

Fig. 3 Distribution of centre-to-centre distances between bubbles on molecules containing more than one bubble. Measurements were made on DNA molecules isolated from *cdc*8 at the restrictive temperature.

experiments with *cdc*21 at the restrictive temperature have shown that these DNA molecules also contain small replication bubbles (T.D.P., unpublished observations).

The appearance of small replication bubbles in the elongation mutant *cdc*8 but not in the initiation mutants *cdc*4 and *cdc*7 cannot be explained by suggesting that *cdc*8 is more leaky at the restrictive temperature than *cdc*4 or *cdc*7. As already discussed, at 36° C, *cdc*7 is considerably more leaky than *cdc* 8. These results, therefore, also imply a qualitative difference in the leakiness of *cdc*7 and *cdc*8 (ref. 10). In *cdc*8 at the restrictive temperature, a high proportion of cells are apparently synthesising DNA at a slow rate of chain propagation (since many of the DNA molecules contain small bubbles). In *cdc*7 at the restrictive temperature, the leakiness of synthesis may be the result of a small proportion of cells which escape the initiation block and replicate their DNA at a normal or nearly normal rate of chain propagation (since very few of the DNA molecules contain DNA replication intermediates).

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Radiological mapping of the ribosomal RNA transcription unit in *E. coli*

THE ribosomal RNA genes of *Escherichia coli* are arranged in tandem behind a common promotor in the order of 16S, 23S, 5S (for review see ref. 1). In this report we redetermine the positions of the 16S (0.55×10^6 dalton), 23S (1.10×10^6 dalton), and 5S rRNA (0.04×10^6 dalton) cistrons on their unit of transcription (scripton) by the ultraviolet light mapping technique[2–9] principally to demonstrate the applicability of this technique to the determination of the transcriptional linkage of genes in *E. coli*.

The ultraviolet light technique for mapping transcriptional linkage is based on the observation that certain lesions induced in DNA by ultraviolet light cause premature termination of transcription at the site of the lesion and that no reinitiation occurs beyond the lesion up to the next promotor[5,6,10,11]. Therefore, the expression of promotor distal genes is more sensitive to ultraviolet irradiation than that of promotor proximal ones.

Rates of synthesis of 23S, 16S and 5S rRNA after irradiation of *E. coli* with various doses of ultraviolet light were determined by ^{3}H-uridine pulses and quantitation of the RNA species after polyacrylamide gel electrophoresis. Errors originating with the isolation procedure were minimised by providing internal standards through prelabelling of the mature rRNAs with ^{14}C-uridine. In Fig. 1 (left and right panels) frames (*a*) display rRNAs synthesised in the unirradiated sample, frames (*b*) to (*f*) (left panel) and (*b*) to (*d*) (right panel) the synthesis in ultraviolet-light-irradiated cultures in the order of increasing dose. As Fig. 1 shows, synthesis of 23S rRNA was reduced more by ultraviolet light irradiation than was the synthesis of 16S rRNA. Synthesis of 5S rRNA was very sensitive to ultraviolet light. A greater reduction in 23S rRNA synthesis compared with 16S rRNA synthesis after ultraviolet irradiation has been observed previously[12–14].

Evaluation of a series of such experiments leads to the dose-effect curves in Fig. 2. The straight line parts of the curves, which extrapolate to 160 and 170% at the ordinate, yield inactivation coefficients of -3.7×10^{-2} s^{-1}, -0.12 s^{-1}, and -0.12 s^{-1} for synthesis of 16S, 23S and 5S rRNA, respectively. The ratio of 16S *versus* 23S rRNA synthesis as a function of dose is given in the insert of Fig. 2. We have utilised this RNA ratio curve in determining the extent of photoreactivation of RNA chain terminating lesions.

Inactivation curves for 16S, 23S and 5S rRNA synthesis all have shoulders in a semi-log plot, but with ultraviolet doses greater than 10 s they all enter into a straight line portion (Fig. 2). From this it can be concluded that all six rRNA transcription units[1] in a single *E. coli* chromosome are structurally identical.

We suggest that the shoulders of the survival curves are caused by the following effect. Not all ribosomal RNA scriptons are transcribed at the maximal rate in the unirradiated cell. As the number of unhit scriptons decreases within a cell with increasing ultraviolet dose, dormant rRNA scriptons become activated, or the remaining rRNA scriptons are utilised better. When cells are irradiated for more than 10 s the rates of rRNA synthesis are exclusively determined by the number of unhit cistrons for the mature rRNA species.

16S, 23S and 5S rRNA can be positioned fairly accurately on p30, the precursor RNA containing the mature rRNA species, if the inactivation coefficients and molecular weights of

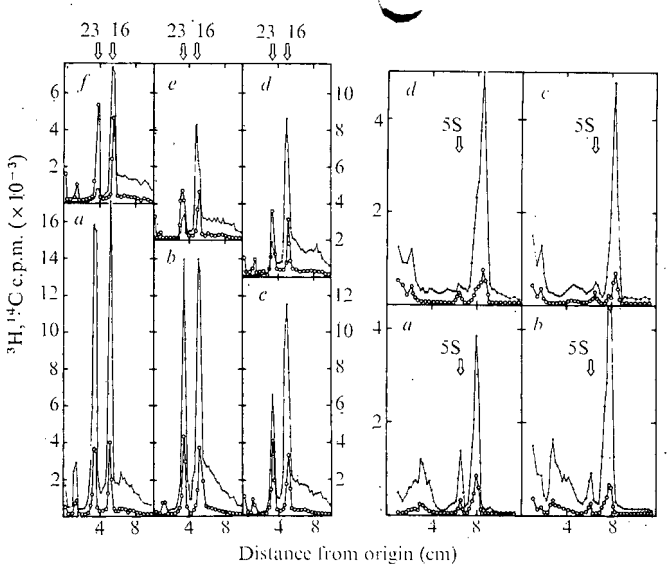

Fig. 1 Left panel: tritiated uridine labelling of 16S and 23S rRNA as a function of ultraviolet irradiation dose. A 20 ml culture of the ultraviolet-light-sensitive strain *E. coli* B_{S-1} (ref. 24) was grown in M-9 medium (M–9 plus 5% (w/v) casamino acids) at 37° C. At a concentration of 0.5×10^8 cells per ml, ^{14}C-uridine (54 mCi mmol^{-1}, Amersham–Searle) was added (0.01 μCi ml^{-1}) and cells were grown for approximately two more generations, about 90 min. This prelabelled culture was chilled on ice, centrifuged (5,100*g*, 15 min), resuspended in 30 ml fresh M–9 medium (approximately 10^8 cells ml^{-1}) and incubated for another 45 min at 37° C. The culture was then chilled on ice, split into six equal 5 ml portions and all but one irradiated at 0° C with a GE G4T4/1 low pressure mercury lamp for various times (incident dose rate at the position of the sample was 14 erg mm^{-2} s^{-1}, the effective dose rate was 10.5 erg min^{-1} s^{-1}). The unirradiated and five irradiated samples were transferred to culture tubes and incubated with vigorous shaking for 5 min. Tritiated uridine (42 Ci mmol^{-1}, Amersham–Searle) was added to a concentration of 1 μCi ml^{-1} to each culture. At 12.5 min after ^{3}H-uridine addition, the cultures were poured into ice cold centrifuge tubes, the cells were pelleted, resuspended in 2 ml 3mM EDTA (*p*H 7.2) and lysed by addition of 0.2 ml 10% Na dodecyl sulphate (SDS, Biorad) in a 1 min, 80° C incubation. RNA was extracted from the lysates with H_2O–saturated phenol, precipitated and resuspended in 0.30 ml 3 mM EDTA. To these RNA preparations sucrose was added to 15% and SDS to 1%. Samples of 0.10 ml of each RNA preparation were layered onto 15 cm 2.6% acrylamide gels and electrophoresed at 70 V for 5.5h; the gels were sliced into 2 mm fractions, digested in peroxide and counted in a liquid scintillation counter. The counts in the ^{3}H–pulse–labelled RNA and ^{14}C–prelabelled RNA were corrected for interchannel spillover. Right panel: tritiated uridine labelling of 5S and 4S RNA as a function of ultraviolet irradiation dose. Samples of 0.10 ml of the same RNA preparations used in the left panel were layered on to 8.0% polyacrylamide gels and electrophoresed at 75 V for 6 h in order to resolve the 5S and 4S RNAs. The major peak to the right of 5S rRNA is the 4S tRNA fraction. In both panels the ultraviolet irradiation times were (*a*) 0 s; (*b*) 8 s; (*c*) 15 s; (*d*) 23 s; (*e*) 31 s; (*f*) 38 s. No tritium counts in the 5S region were detectable at irradiation times in excess of 23 s (data not shown). ●, ^{3}H c.p.m.; ○, ^{14}C c.p.m.

the rRNAs are known. Since synthesis of the 23S is 3.25 times more sensitive than that of 16S rRNA, its 3′ terminus must be located at least $3.25 \times 0.55 \times 10^6$ daltons $= 1.80 \times 10^6$ daltons away from the p30 promotor. Since the molecular weight of the 5S rRNA is 0.04×10^6 and since 5S synthesis has essentially the same inactivation coefficient as 23S (Fig. 3), 5S must follow the 23S very closely on the transcriptional map. Thus, by converting ultraviolet-light-inactivation coefficients into molecular weights, using the molecular weight of 16S rRNA as a standard, and assuming the 16S RNA cistron to be adjacent to the promotor locus, we project a molecular weight of about 1.85×10^6 for p30. This size is close to previous determinations of the p30 molecular weight between 1.75 and 10^6 (ref. 15) and 1.85×10^6 (ref. 16). If this size for p30 is correct, our data would locate the 16S, 23S and 5S rRNA as shown in Fig. 3*a*, with the 3′ terminus of p30 at about 1.85×10^6 daltons. If the molecular weight of p30 were about 2.1×10^6 (ref. 17) the 16S rRNA could be preceded by approximately 0.09×10^6 daltons of non-conserved spacer RNA and the second spacer situated between 16S and 23S would be about 0.30×10^6 daltons (Fig. 3*b*). Alternatively, the additional 0.25×10^6 daltons of non-conserved RNA could be located beyond the 3′ end of the map as drawn (dashed line) in Fig. 3*a*. Figure 3*a* and *b* gives the extreme positions of 16S, 23S and 5S rRNA on p30. Any intermediate position that places the 3′ end of the 23S at 3.25 times the distance of the 16S 3′ end from the promotor would be consistent with our data.

From the inactivation coefficient for the synthesis of 16S rRNA, the dose required to produce 1 transcription terminating

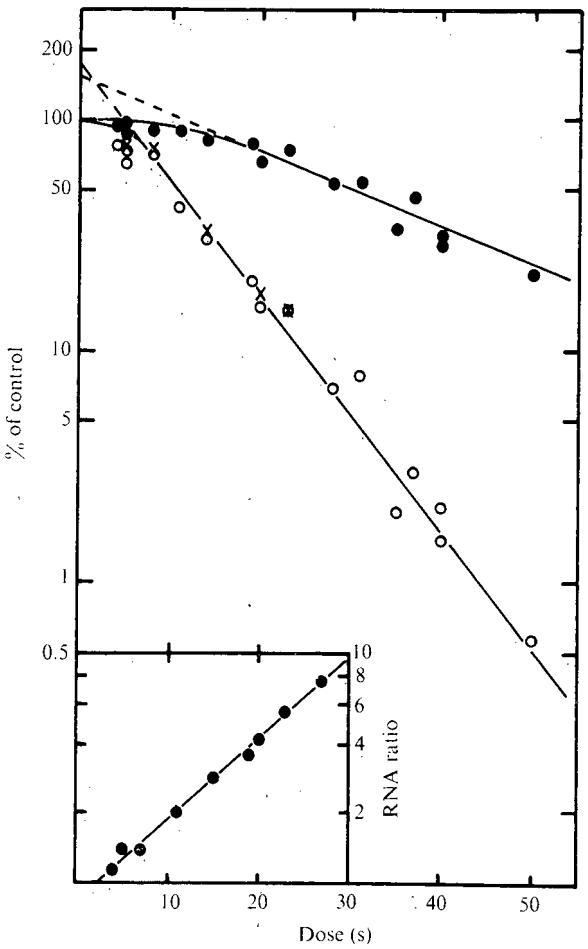

Fig. 2 Reduction of synthesis of rRNA species as a function of ultraviolet light dose. Data are taken from several experiments of the type shown in Fig. 1. The relative amount of 23S, 16S (converted from 17S values) and 5S rRNA can be calculated accurately, using the ^{14}C–prelabelled rRNA as an internal standard, from the expression:

$$\%\text{ of control synthesis} = \left[\frac{^3\text{H c.p.m. RNA}_x}{^{14}\text{C c.p.m. RNA}_x}\right]_d \cdot \left[\frac{^{14}\text{C c.p.m. RNA}_x}{^3\text{H c.p.m. RNA}_x}\right]_{\text{control}}$$

where x refers to the species of rRNA under consideration, the RNA in the brackets subscribed with a d is from a culture irradiated with a dose d, and the RNA in the brackets subscripted with 'control' is from the unirradiated culture. Background radioactivity due to non-rRNA was subtracted from the total radioactivity in the peak fractions for 23S, 17S and 5S rRNA. The exponential slopes, determined from least squares analyses of all points greater than 10 s, were $K_{16} = -3.7 \times 10^{-2}$ s^{-1}, $K_{23} = K_5 = -0.120$ s^{-1}. The ordinate intercepts of the exponential portions of the RNA synthesis dose-effect curves for 16S and 23S rRNA were 160% and 170% respectively. ●, 16S rRNA; ○, 23S rRNA; ×, 5S rRNA. The inset shows the increase in 16S rRNA synthesis relative to 23S rRNA synthesis (the RNA ratio) with increasing ultraviolet light dose.

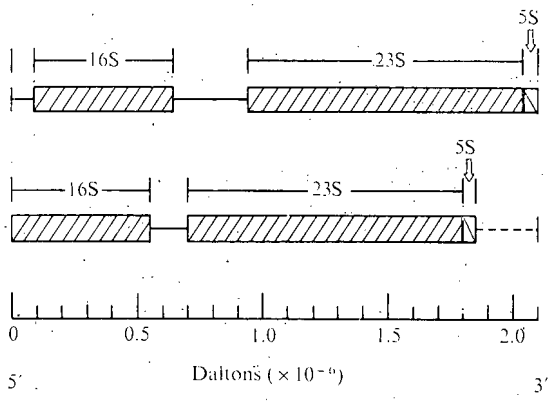

Fig. 3 Map of the *E. coli* ribosomal RNA transcription unit determined by radiological mapping. Two possibilities for the arrangement of the rRNAs (hatched areas) and non-conserved spacer RNAs (solid or dashed lines) are shown; model *a* assumes the molecular weight of p30 is 1.85×10^6 (refs 15 and 16) and model *b* assumes the molecular weight is 2.1×10^6 (ref. 17).

lesion per 1,000 DNA base pairs in the p30 scripton was determined to be between 550 and 640 erg mm^{-2}.

From the extent to which ultraviolet-light-induced transcription terminating lesions in DNA can be eliminated by photoreactivation, one can estimate what fraction of these lesions comprises pyrimidine dimers. After maximal photoreactivation; 70% of the transcription terminating lesions were repaired as determined from the shift in the ratios of 16S to 23S rRNA (ref. 7 and inset, Fig. 2). This suggests that at least 70% of the transcription terminating lesions comprises pyrimidine dimers[18]. Photoreactivation of ultraviolet-light-induced lesions which impair RNA synthesis in general has been reported earlier[19].

Actinomycin D has been used in studies of transcriptional linkage[20,21] with the same rationale as we use in the ultraviolet light mapping technique. The ribosomal genes of *E. coli*, however, were incorrectly assigned to separate transcription units through the actinomycin D technique[20], and the transcriptional linkage of the β-galactosidase and transacetylase genes of the *lac* operon of *E. coli* (ref. 22), was determined correctly only through an error in evaluating the experimental data. The transcriptional arrangement of the rRNAs in mouse L cells was incorrectly inferred from actinomycin D inhibition of RNA synthesis (see ref. 7). Thus far, actinomycin D has failed as an agent for transcription mapping in both prokaryotes and eukaryotes[7], whereas ultraviolet light mapping has been used successfully in both systems (for review see ref. 23).

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Distribution of DNA in dividing spinach chloroplasts

SPINACH chloroplasts, like other higher plant chloroplasts, contain DNA[1] and divide[2]. It has been estimated[3-5] that chloroplasts contain many copies of DNA, but in higher plants, which contain large numbers of chloroplasts per cell[2,6] it is not known when the DNA of chloroplasts replicates and how it is distributed when they divide. In the unicellular alga, *Ochromonas*, which contains a single chloroplast, ^{3}H-thymidine is readily incorporated into chloroplast DNA and is distributed equally among the progeny chloroplasts[3]. On the other hand, in *Acetabularia*, which contains large numbers of chloroplasts per cell, DNA could not be detected in 65–80% of the plastids, but could easily be detected in the remainder[7].

Disks of spinach leaves grown in sterile culture were used to study the distribution of DNA in dividing chloroplasts in both pulse and pulse/chase experiments. In this system chloroplast number per cell increases 5–10-fold over a 7 d culture period in the light[2]. There is virtually no cell division during this time[2].

Using ^{3}H-thymidine and autoradiography on EDTA-separated cells dried on to glass slides (Fig. 1) we found that in spinach leaf discs both chloroplast and nuclear DNA synthesis continues during cell expansion. The specificity of incorporation into DNA was established using DNase, RNase and acid extractions[8].

The distribution of label amongst daughter chloroplasts was followed in freshly excised spinach disks (2 mm dia-

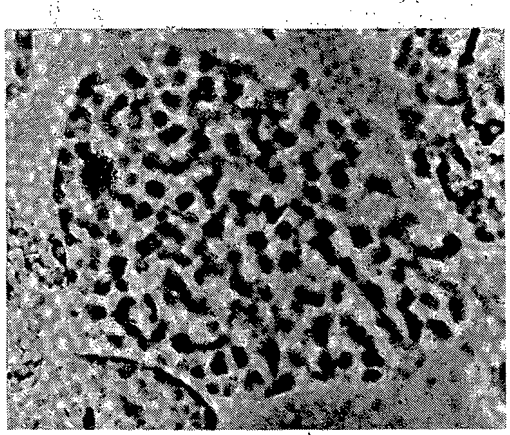

Fig. 1 Autoradiograph of leaf cell showing ^{3}H-thymidine incorporation. Tissue was incubated in ^{3}H-thymidine for 24 h and chased 4 d as indicated in Table 1 (×400).

Table 1 ^{3}H-Thymidine incorporation into spinach chloroplasts

	End of pulse	End of chase
Grains per chloroplast	23.9	4.5
Labelled chloroplasts	100%	99%
Chloroplasts per cell	23	148

Spinach disks 2 mm in diameter were excised from the base of leaves 2 cm long and grown on sterile agar[4]. 1 ml of 50 μCi 6-^{3}H-thymidine (27 Ci mM^{-1}, The Radiochemical Centre, Amersham, UK) was added under sterile conditions to the 20 ml of medium in 9 cm Petri dishes. The tissue was incubated for 24 h (14 h d^{-1}, 20,000 l×) in a growth cabinet. After incubation, nine disks were fixed in 3% glutaraldehyde after a rinse in 2.07 mM cold thymidine. The other nine disks were transferred to fresh medium (see text), containing approximately a 1,000 times excess of cold thymidine. After 4 d in the fresh medium, the nine remaining disks were fixed in 3% glutaraldehyde. The disks from both the pulse and chase were treated with 0.05 M EDTA (*p*H 9) overnight at 60° C to separate the cells. The cells were spread on to slides, air dried and dipped in Ilford L4 nuclear emulsion. The slides were exposed 13 d at 5° C before developing and mounting as permanent slides. The data for the pulse was obtained from 10 cells with all plastids scored as labelled or unlabelled, and 10 plastids from each cell scored for grain number. The chase data was obtained in the same way except that 20 plastids per cell were scored for grain number. Independent counts were made by two observers and only chloroplasts clearly separated from each other were scored. Background corrections were made; these varied from zero to two grains per chloroplast.

meter) incubated in ^{3}H-thymidine for 24 h. At the end of the incubation period, one half of the disks were collected for autoradiography, and the other half rinsed with sterile water and transferred to fresh medium containing an excess of cold thymidine for a further 4 d growth period.

At the end of the ^{3}H-thymidine pulse there were 23 plastids per cell and this increased to 148 at the end of the chase (Table 1). This represents approximately 2.7 generations. Almost all plastids were labelled both at the end of the pulse and at the end of the chase (Table 1). These results indicate that the label is distributed to virtually all daughter chloroplasts. The mean number of grains decreased from 23.9 at the end of the pulse to 4.5 at the end of the chase (Table 1), which is close to the 3.7 expected if the grain number was halved at each generation. This is consistent with the chloroplast DNA being conserved during the chase period. The nuclear DNA also appears to be largely conserved as there were 44% labelled nuclei at the end of the pulse and 36% at the end of the chase. We have also found a fourfold increase in the total DNA of discs, over a 6 d growth period, even though there is no significant cell division during this time.

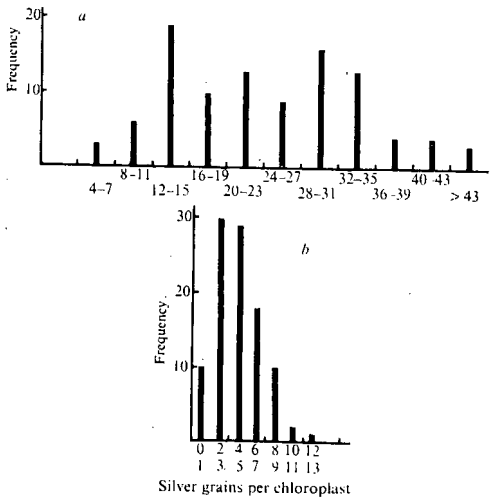

Fig. 2 Frequency distribution histograms of silver grains over a 100 chloroplasts after, *a*, a 24-h pulse in ^{3}H-thymidine, and, *b*, a 4-d chase as indicated in Table 1. The observed distribution of the pulse differs from that of the chase. The distribution of the chase does not differ significantly from a Poisson distribution having the same mean (chi-square test: $P > 0.05$). An autoradiograph of a cell after the chase is shown in Fig. 1.

The distribution of silver grains at the end of the pulse probably reflects the different times at which plastids completed their last division (Fig. 2). No highly labelled chloroplasts were found at the end of the chase and the labelling pattern corresponds closely to a Poisson distribution. This is good evidence that the label distributes almost equally amongst daughter chloroplasts as is the case in *Ochromonas*[3].

Our results differ from those of Woodcock and Bogorad[7] with *Acetabularia* where there are large variations in the DNA content of chloroplasts. Herrmann[9,10] has found, using chloroplast preparations, isolated from whole or parts of *Beta vulgaris* leaves, that large chloroplasts contain more DNA than small chloroplasts. He did not look at variations in the DNA content of chloroplasts in single cells. Some of the variation in DNA amongst chloroplasts of different sizes can be related to developmental and nuclear genotype changes. We consider these differences are unrelated to the segregation of DNA that occurs in a rapidly dividing chloroplast population which is concomitantly synthesising DNA.

The data we have obtained establish that there is active chloroplast DNA synthesis in expanding leaf cells of spinach and that chloroplast DNA segregates to all daughter chloroplasts during chloroplast replication. The data further suggest that all chloroplasts of the cell divide rather than a small subpopulation as has been proposed from chloroplast size and observations[6].

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Friend virus release and induction of haemoglobin synthesis in erythroleukaemic cells respond differently to interferon

Experiments with intact cells have provided the firmest evidence that the synthesis of viral proteins is the main function inhibited in cells treated with interferon. Changes

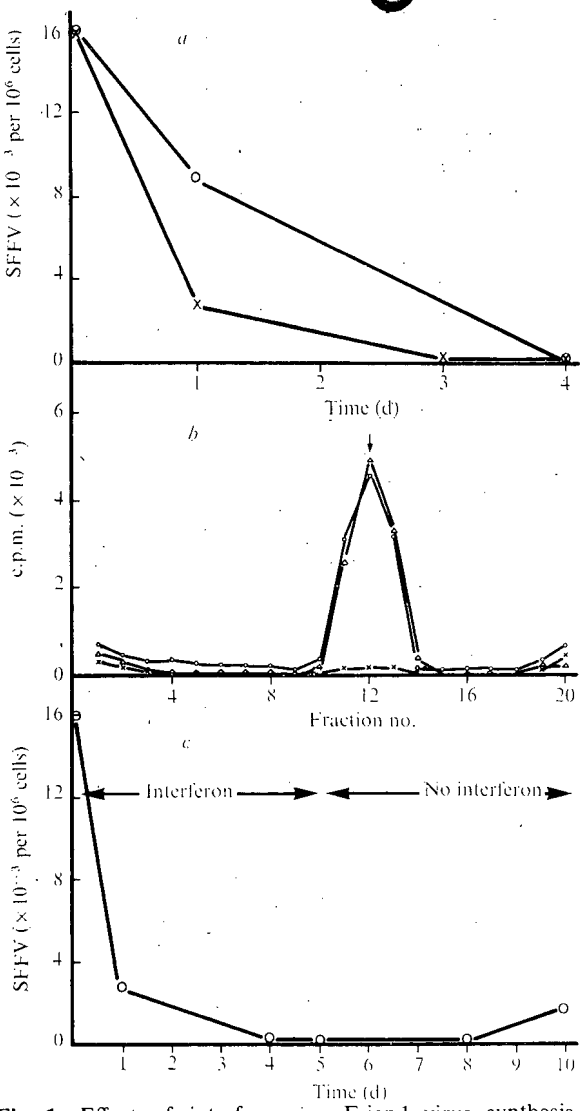

Fig. 1 Effect of interferon on Friend virus synthesis in unstimulated FSD1/F4 cells. Mouse interferon was prepared by the infection of L-929 cells with Newcastle disease virus (NDV-California strain). Cell supernatants were dialysed against 0.1 M glycin—HCl buffer (*p*H 2.0) for 5 d and further purified by gel chromatography[15]. The highly purified samples of mouse interferon (10^7 mouse reference research standard units per mg protein) were assayed by a plaque reduction test of vesicular stomatitis virus in mouse L-929 cells[15]. FSD1/F4 cells have been cultured as described previously[6] at a density of 1.0×10^6 cells ml^{-1}. Medium was removed by centrifugation and cells transferred to the same volume of either fresh medium or fresh medium containing 300 or 900 U ml^{-1} mouse interferon, respectively. Cells were transferred in daily intervals to fresh medium with or without interferon. After removal of the cells, aliquots of cell supernatants were assayed for SFFV in DBA-2 mice[16]. Total synthesis of C-type particles was analysed by measurement of ^{3}H-uridine incorporation into cell supernatant particles banding at 1.16–1.18 g cm^{-3}. Cells were exposed to either 600 U ml^{-1} mouse interferon or 500 U ml^{-1} chicken interferon from 0–48 h and 25 μCi ml^{-1} 5,6 ^{3}H-uridine (Amersham; specific activity, 50 Ci $mmol^{-1}$) from 24–48 h. Cell supernatants were cleared from debris at 8,000*g*, 10 min and centrifuged at 60,000*g* 1 h 4° C. Pellets were resuspended in a buffer containing 0.1 M NaCl, 0.01 M Tris-Cl, 0.001 M EDTA, *p*H = 7.4, layered on top of a 15–60% (w/v) sucrose gradient in the same buffer and centrifuged for 75 min in a Spinco SW 50-1 rotor at 42,000 r.p.m. Acid precipitable radioactivity was determined in each fraction. *a*, Reduction of Friend virus (FV) release by interferon. Spleen focus formation of supernatant particles. ○, 300 U ml^{-1} interferon; ×, 900 U ml^{-1} interferon. *b*, Reduction of FV release by interferon, uridine incorporation into viral particles, density 1.16–1.18 g cm^{-3} (↓), sucrose gradient. ○, Untreated cells; ×, mouse interferon 600 U ml^{-1}; △, chicken interferon 500 U ml^{-1}. *c*, Aftereffect of interferon treatment. 900 U ml^{-1} treatment as in *a*. Interferon-free medium from day 5 on.

in an interferon-treated cell which lead to the development of resistance may be reflected also in a cell-free system for protein synthesis. Thus, polyribosomes of interferon-treated L cells were found unable to translate either mengovirus mRNA or haemoglobin mRNA—in contrast to the endogenous mRNA synthesis which was not impaired[1]. A similar inhibition of the translation of exogenous viral or cellular mRNAs was reported for cell-free extracts from interferon-treated Ehrlich ascites cells[2]. These observations raise the question of whether the expression of some cellular functions is as equally affected as that of viruses. The inhibition of haemopoietic colony growth and of phytohaemagglutinin stimulation of lymphocytes by interferon seems to indicate that interferon does have such a dual effect[3,4]. We show here, however, that interferon inhibits virus release or replication but that it does not interfere with DMSO-induced differentiation.

Friend virus-transformed erythroleukaemic spleen cells can be maintained in culture. They can be induced to differentiate[5] and to synthesis haemoglobin[6]. Some of the erythroleukaemic cell lines (clone FSD1/F4) liberate biologically active Friend virus (FV), that is, spleen focus forming virus (SFFV) (ref. 7) and lymphatic leukaemia

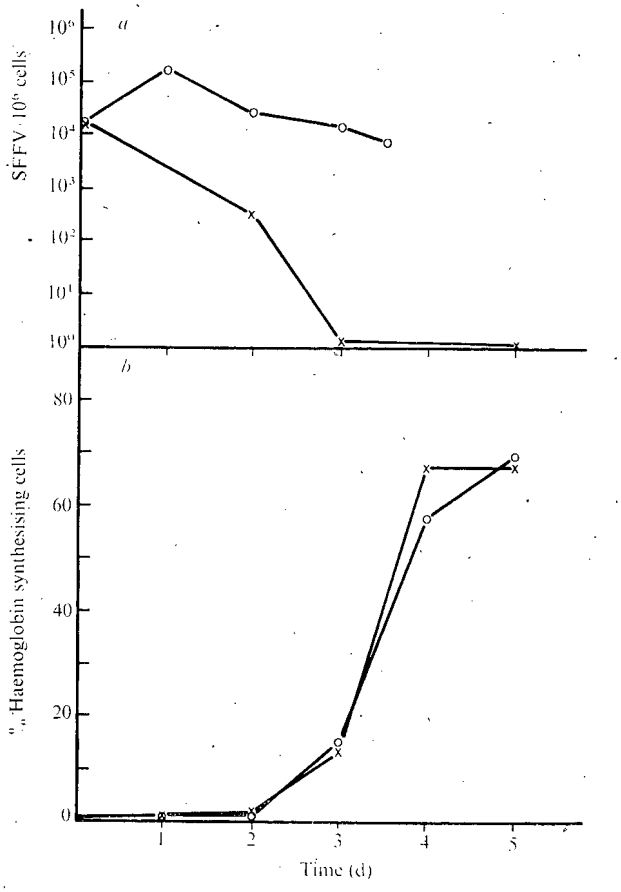

Fig. 2 Effect of exogenous interferon on the production of FV (SFFV) and on haemoglobin synthesis during dimethylsulphoxide (DMSO)-induced differentiation of FSD1/F4 cells. Stimulation of FSD1/F4 cell cultures by DMSO treatment has been described[6]. Interferon was added to the culture medium at a concentration of 900 U ml^{-1}. During the course of stimulation, cells were transferred daily to fresh medium containing 1.5% DMSO with or without interferon. The production of SFFV was tested at various intervals during stimulation as described previously[16]. Erythroid differentiation of cells was estimated by staining cells with benzidine. *a*, Effect of interferon on virus synthesis in DMSO stimulated cells. ○, DMSO 1.5%, no interferon; ×, DMSO 1.5%, 900 U ml^{-1} mouse interferon. *b*, Lack of effect of interferon on haemoglobin synthesis. The % of benzidine-positive cells is plotted. ○, DMSO 1.5%, no interferon; ×, DMSO 1.5%, 900 U ml^{-1} mouse interferon.

virus (LLV). LLV acts as a helper in spleen focus formation. This cell line can therefore be used to study the effect of exogenous homologous (mouse) interferon on the release of C-type viruses and on the induction of erythroid differentiation (haemoglobin synthesis).

Interferon inhibits tumour formation by RNA tumour viruses[8,9]. In some instances, however, interferon can be used to enhance neoplastic development[10]. Interferon also interferes with the transformation of fibroblasts by sarcoma viruses[11]. Mice with Friend erythroleukaemia have been reported to display a reduced capacity to respond to inducers of interferon[12,13]. No interferon could be detected in the supernatants of FSD1/F4 cells or two other lines of SFFV-transformed cells when proliferation was either in the absence or presence of dimethylsulphoxide (DMSO), indicating that FV does not trigger the interferon response in these cells. Although interferon production can be induced in these cells by an exogenous viral inducer (Newcastle disease virus) no response to poly(I) · poly(C) (ref. 12) was observed (P.S. and W.O., unpublished). Similar observations on differential inducibility of interferon in transformed mouse cells were made by others (F. Ruddle, unpublished).

The effect of interferon on FV release in unstimulated cells was demonstrated by the addition of 300–900 mouse reference research standard units (U) interferon ml^{-1} to cell clone FSD1/F4. We find a more than 100-fold reduction both of spleen focus formation with cellular supernatants and of uridine label in C-type particles. (Fig. 1*a*, *b*). Heterologous chicken interferon does not reduce the virus yield (Fig. 1*b*). The recovery of virus production after interferon treatment for 5 d and then the omission of interferon is slow and possibly incomplete. Five days after the omission of interferon only 10% of the normal virus level is obtained (Fig. 1*c*). The growth rate of the interferon treated cells was only slightly reduced.

When FSD1/F4 cells are exposed to growth medium which contains 1–1.5% DMSO, not only the onset of erythroid differentiation but also a 5–10-fold increase in released virus particles during the first and second day of differentiation can be observed. The titre of released virus increases 10–100-fold as assayed either by the XC-test (LLV) or by the spleen focus forming test (LLV+SFFV)[7]. Interferon, however, depresses the virus synthesis below background level (Fig. 2*a*). Differentiation as measured by globin synthesis is only slightly inhibited by 300–900 U ml^{-1} interferon. If interferon is added 3 d before the onset of DMSO induced differentiation, perfectly normal differentiation is observed. The course of differentiation, that is the number of benzidine positive cells after adding DMSO or DMSO and interferon, remains unchanged (Fig. 2*b*). The amount of globin synthesised after 4 d stimulation with DMSO is not reduced by adding 300–900 U ml^{-1} interferon (Fig. 3).

As the degree of purity of interferon often reflects on the meaningfulness of results we confirmed our data obtained with partially purified interferon (1.5×10^5 U mg^{-1} protein), by using a highly purified interferon preparation (10^7 U mg^{-1} protein). We found that within our limits the degree of purity had no effect on the interferon action.

The data above make possible, for the first time, the differentiation between the effect of exogenous interferon on C-type virus release in transformed cells and the effect on induced differentiated cellular functions in the same cell. While virus synthesis is strongly inhibited, differentiation remains unaffected. Interferon neither changes the time course nor the amount of haemoglobin synthesis. It does not change the number of cells involved in haemoglobin synthesis either. We conclude that the release of Friend virus is not required for *in vitro* erythroid differentiation of these cells.

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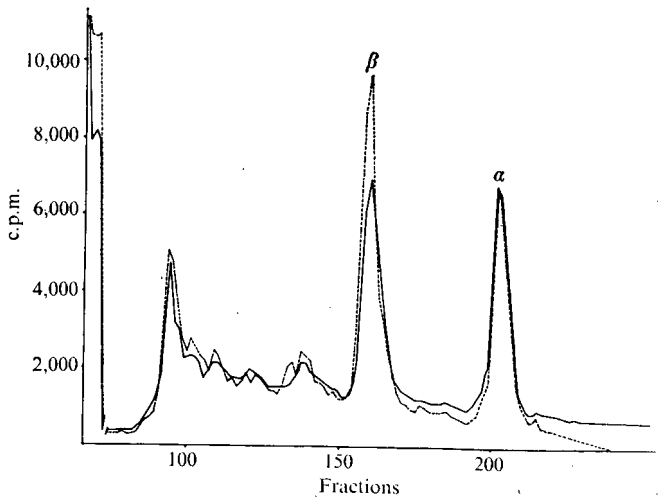

Fig. 3 Stimulation of globin synthesis with 1.5% DMSO; Effect of interferon. Cells were pretreated with interferon 900 U ml^{-1} for 3 d. DMSO (1.5%) was then added and incubation with DMSO+interferon continued for another 4 d. Medium was replaced daily. Cells were labelled with ^{3}H- or ^{14}C-leucine in leucine-free medium for 16 h. Extraction of NP40 soluble cytoplasmic proteins and separation of globin chains on carboxy methylcellulose columns was done as described previously[6]. ----, DMSO 1.5% no interferon, ^{14}C-leucine; ———, DMSO 1.5% 900 U ml^{-1} mouse interferon, ^{3}H-leucine.

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Erythropoietin responsiveness of differentiating Friend leukaemia cells

INCLUSION of dimethylsulphoxide (DMSO) in the media of suspension cultures of Friend leukaemia cells (FLC) results in the erythroid differentiation of more than 50% of the cells[1]. This differentiation is demonstrated by morphological and biochemical alterations which are characteristic of normal erythroid maturation[2-5]. We report here that the differentiation of these leukaemic cells is accompanied by the development of responsiveness to erythropoietin (Ep), the physiological regulator of erythropoiesis.

Friend leukaemia cells (clone 745A) were grown in suspension culture in media consisting of basal Eagle's medium with Earl's balanced salts (GIBCO), made 15% with foetal calf serum (GIBCO) at 37° C in a humidified atmosphere of 5% CO_2 and 95% room air. The cultures were passaged at 3 or 4 d intervals at a seeding concentration of 1×10^5 cells ml^{-1}. DMSO (Fisher Sci.) was added (2% v/v) where appropriate at the time of seeding the cultures. Erythropoietin (human urinary erythropoietin 75.4 IU mg^{-1}) was added to the suspension cultures at the times indicated.

To assess the rate of haem synthesis, 0.5 μCi of mouse transferrin-bound ^{59}Fe was added to culture plates containing 3 ml of the suspension culture and after 4 h further incubation the cells were collected, washed twice with isotonic saline and the haem extracted with cyclohexanone according to the method of Krantz[6], and counted. This procedure provides an accurate assessment of the rate of haem synthesis by Friend leukemia cells[7]. We have already shown that during DMSO-induced differentiation of Friend erythroleukaemia cells, increased iron incorporation into haem was coordinated with increases in the rate of synthesis of globin, haemoglobin, and globin mRNA[8]. To assess the rates of DNA, RNA and protein synthesis, 5 μCi of ^{3}H-thymidine, ^{3}H-uridine or ^{3}H-leucine (New England Nuclear Co.) were added to appropriate culture plates containing 4 ml of suspension cell and after 40 min of incubation the cells were collected, washed twice, and the rate of precursor incorporation determined according to the method of Schneider as discussed by Shatkin[9]. All radioactive assays were determined using three plates for each experimental point and each experiment was carried out at least three times.

Ep was added to suspension cultures of erythroleukaemia cells which had previously been grown in the presence of DMSO for 3 d (Table 1). This significantly stimulated the rate of haem synthesis of the cells. Ep had no significant effect on the rate of increase in cell numbers in the culture (which increased from $0.95\pm0.03\times10^6$ cells ml^{-1} on day 3 to $1.24\pm0.04\times10^6$ cells ml^{-1} on day 4) nor did it result in an increase in the proportion of benzidine-positive cells in the culture. Ep had no effect on the rate of haem synthesis by cells which had not been cultured in the presence of DMSO (Table 2).

The stimulatory effect of Ep on DMSO-treated cells was not limited to haem synthesis. The effects of Ep on the rate of precursor incorporation into DNA, RNA and protein are given in Table 2. The increases in leucine and uridine incorporation were quantitatively similar to that observed for ^{59}Fe; the rate of DNA synthesis also increased. These effects of Ep on the rates of macromolecular synthesis by these differentiating leukaemic cells were similar to those reported for normal rat bone marrow cells[10] and foetal liver erythroid cells[11]. Although Ep had no effect on the rates of haem, RNA or protein synthesis by cells which had not been cultured in the presence of DMSO, the addition of Ep to suspension cultures of these cells resulted in a decrease in the rate of thymidine incorporation into DNA.

During cell growth and replication, Ep responsiveness could be reliably detected when Ep was added on the third day of culture and haem synthesis assayed 1 d later. The addition of Ep earlier during culture with assay of haem synthesis 24 h after addition produced no significant effect. The addition of Ep to cultures which had been growing in the presence of DMSO for more than 3 d also resulted in an increase in the rate of haem synthesis. By contrast, cultures to which Ep was added at the time of seeding in the presence of DMSO synthesised less haem on the fourth day of culture than cultures also seeded in the presence of DMSO but to which Ep was not added. In six such experiments the rate of haem synthesis (mean c.p.m. per plate ± s.e. for 17 degrees of freedom) was 16±4.8% less than cultures seeded without Ep.

Table 1 Effect of Ep on erythroleukaemic cells grown in DMSO medium

Parameter	Mean % stimulation ± s.e.m.	No. experiments
(1) Haem synthesis		
a CPM-^{59}Fe per plate	+29 ± 4.6	29
b CPM-^{59}Fe per 10^6 cells	+29 ± 4.1	29
(2) Cell number	+3 ± 2.4	29
(3) % Benzidine-positive cells	+6 ± 4.7	20

Tissue culture plates were set up containing 4 ml of suspension culture which was made 2% (v/v) with DMSO at the time of seeding. After 3 d of culture Ep (0.06 IU ml^{-1}) was added to appropriate plates and 24 h later, after removal of 1 ml from each plate for cell counts and morphological studies, ^{59}Fe was added and 4 h later the haem extracted. For morphological studies, slides were made with a cytocentrifuge, were benzidine stained[13], and counterstained with Wright-Giemsa. The proportion of benzidine-positive cells was determined by a single observer in a single-blinded position. The following equation was used to determine % stimulation of each parameter:

$$\frac{\text{(Parameter Ep culture)} - \text{(Parameter no Ep culture)} \times 100}{\text{(Parameter no Ep culture)}}$$

The % stimulation was determined for each individual experiment and the data so derived pooled and used to calculate the grant mean and s.e.m. for each parameter.

Ep was an effective stimulant of haem synthesis within a broad range of concentrations. A comparison of the stimulatory effects of 0.04 IU ml^{-1} and 0.2 IU ml^{-1} on the rate of haem synthesis revealed no significant difference (34.5±6.4% stimulation for 0.04 IU ml^{-1} compared with 44±6.6% stimulation for 0.2 IU ml^{-1}).

We have tested three different human urinary Ep and three different Step III sheep plasma Ep and have found no significant differences in their effects.

In a preliminary communication we reported that Ep stimulated the rate of haem synthesis by Friend leukaemia cells which had previously been cultured in the presence of DMSO[12]. In those studies, however, the condition of culture were such that the cells were not replicating and the rate of haem synthesis in all the cultures was declining. The studies reported here demonstrate that under conditions of growth the erythroid differentiation of these leukaemic cells is accompanied by the development of responsiveness to Ep. It is the process of differentiation *per se* and not the penetrant carrier properties of DMSO, that is responsible for the responsiveness to Ep, since Ep stimulated the rate of haem synthesis when the differentiating cells had been transferred to fresh medium before adding Ep[12]. It is of interest in this regard that under the conditions of culture reported here, the appearance of responsiveness to Ep correlated with a major increase in the proportion of recognisable differentiating cells in the culture (the percentage of benzidine-positive cells on days 1 and 2 of culture were 1% and 5%, whereas on the third day it was 50%). This suggests that under these conditions Ep affects cells which have differentiated to the point of having

synthesised enough haem to be recognisable by benzidine staining. The effects of Ep on normal erythroid precursor cells which are more differentiated than the committed erythroid stem cell have been reported by others [13-15]. The apparent inhibitory effects of Ep on DNA synthesis by control cells and on the rate of haem synthesis by DMSO-treated cells when Ep was added at the time of seeding was unexpected and may have been due to other substances present in the preparation of human urinary Ep. The resolution of this question awaits the availability of pure Ep.

Table 2 Effects of Ep on synthesis of DNA, RNA and protein

Macromolecule	Ep	DMSO-containing cultures Mean c.p.m. per 10^6 cells ± s.e.	Control cultures Mean c.p.m. per 10^6 cells ± s.e.
(*a*) DNA	0	6,073 ± 1182	3,928 ± 158
	+	7,474 ± 161	1,501 ± 34
(*b*) RNA	0	1,737 ± 145	5,018 ± 254
	+	2,556 ± 174	5,381 ± 137
(*c*) Protein	0	4,408 ± 84	15,687 ± 310
	+	6,653 ± 557	15,553 ± 428
(*d*) Haem	0	1,169 ± 128	7
	+	1,796 ± 103	7

Suspension cultures were set up in bottles containing 40 ml each. After 3 d of culture Ep was added to appropriate bottles (0.06 IU ml^{-1}) and after 24 h of additional culture, the suspension cultures were distributed into triplicate plates for determination of the rate of macromolecular synthesis. The differences between the rates of macromolecular synthesis between Ep and non-Ep containing cultures were statistically significant ($P < 0.05$) for all of the DMSO-containing cultures and for the decrease in the rate of DNA synthesis of the control cultures.

All determinations were done in triplicate.

These observations suggest that erythroleukaemic cells, and perhaps leukaemic cells in general, possess the genetic information needed for the development of responsiveness to normal physiological regulators and that under appropriate conditions this information is expressed. Responsiveness to Ep by a continuous suspension culture cell line promises of facilitate studies of the mechanism of action of this hormone.

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Growth of rust fungi of wheat and flax on chemically-defined media

THE rust fungi cause serious economic losses of wheat and other crops. In nature, these organisms complete their life cycles only on living tissues of their hosts and, until recently[1], they have been regarded as obligate parasites due to their failure to grow in axenic culture on non-living substrates. We have now obtained highly reproducible vegetative growth and sporulation of the flax rust fungus, *Melampsora lini* (Ehrenb.) Lév. (race 3) and vegetative growth of two North American races (15B-4 and 56) of the wheat stem rust fungus, *Puccinia graminis* f. sp. *tritici* (Erikss. and Henn.) on chemically defined liquid media, sterilised by millipore filtration and seeded with uncontaminated uredospores. Vegetative growth and some sporulation were also obtained with an Australian isolate of wheat rust, race 126-ANZ 6, 7.

Williams *et al.*[2] reported the first successful establishment of axenic colonies of the wheat stem rust fungus, race 126-ANZ 6,7, starting with uncontaminated uredospores seeded on to an agar medium containing glucose, mineral salts, Evans's peptone and yeast extract. Subsequently much progress has been made in the axenic culture of a number of rust fungi belonging to the *Pucciniaceae*[4-7] and *Melampsoraceae*[8-10]. Nevertheless the results obtained suffer from a number of defects. Most experimenters have employed agar media containing complex organic substances like peptone yeast extract and bovine serum albumin. Only a few attempts[11-13] have been made to grow rusts on chemically defined media containing amino acids and growth on such media has not been equivalent to that obtained in the presence of peptone, yeast extract or bovine serum albumin. Moreover, all the results reported thus far indicate that sporulation in axenic culture is erratic, and there are only two reports in which uredospores produced in axenic culture have been shown to be capable of reinfecting the host[3,6]. Finally, the use of agar based media, sterilised by autoclaving, is itself a disadvantage, since the exact composition of the sterilised medium is not known. Our objectives were to develop chemically defined media (see Table 1 legend) in order to examine the factors controlling sporulation and to compare the amino acid requirements of the wheat and flax rust fungi.

Uncontaminated uredospores of both rusts were raised on susceptible hosts as described elsewhere[14]. All media were sterilised, after the addition of amino acids, glutamine and glutathione as indicated in Table 1, by millipore filtration (pore size 0.22 μm). No agar was employed. The *p*H of all media was adjusted to 5.0. Uredospores (1,000–2,000 mm^{-2}) were floated on the liquid surface (10 ml) in 50 ml flasks. For each treatment five replicate flasks were incubated without shaking at 17° C in the dark. The growth and development of the rust colonies were observed over a period of two months.

The chief results are summarised in Table 1. Luxuriant growth of *M. lini* was obtained on medium A with 45 mM aspartic or glutamic acid, plus cysteine (4.6 mM) and reduced glutathione (3.3 mM), or with 45 mM aspartic or glutamic

Table 1 Comparative behaviour of flax and wheat stem rust fungi *in vitro*

Fungus	Medium	Organic nitrogen source (mM) Aspartic acid*	Glutamine	Cysteine	Glutathione	Growth	Sporulation
Melampsora lini	A	45	0	4.6	3.3	Luxuriant	Frequent
Race 3	A	45	0	4.6 (or 2.3)	0	Luxuriant	Frequent
	A	0	42	4.6	0	Luxuriant	Nil
Puccinia graminis tritici	B	45	0	4.6	3.3	Luxuriant	Infrequent
Race 126-ANZ 6, 7	C	45	0	4.6 (or 2.3)	0	Luxuriant	Infrequent
	C	0	42	4.6	0	Luxuriant	Nil
	C	0	6	4.6	0	Poor	Nil
Race 15B-4 and Race 56	B	45	0	4.6	3.3	Nil	Nil
	C	45	0	4.6 (or 2.3)	0	Nil	Nil
	C	0	42	4.6	0	Luxuriant	Nil
	C	22.5	21	4.6	0	Luxuriant	Nil
	C	0	42 (Asp 45)	4.6	0	Luxuriant	Nil

*Aspartic acid can be replaced by equimolar glutamic acid with identical results. Asp 45 denotes that after 3 weeks' growth 42 mM glutamine was replaced by 45 mM aspartic acid. Basic components of media (l^{-1}): medium A: Sucrose, 50 g; $Ca(NO_3)_2 \cdot 4H_2O$, 2.0 g; KNO_3, 0.25 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; KH_2PO_4, 0.25 g; K_2HPO_4, 0.75 g; NH_4NO_3, 20 mg; 0.8 ml micronutrient solution (containing 13% NaFe (Geigy), 10 g; $MnSO_4 \cdot 7H_2O$, 447 mg; KI, 10 mg; $NiCl_2 \cdot 6H_2O$, 18 mg; $Ti(SO_4)_2 \cdot 9H_2O$, 42 mg; $ZnSO_4$, 35 mg; $CuSO_4 \cdot 5H_2O$, 15 mg; $BeSO_4$, 20 mg; H_3PO_4 (85%), 10 mg; H_2SO_4 (concentrated), 0.2 ml; all in 200 ml glass distilled water). Medium B: glucose, 30 g; $NaNO_3$, 2.0 g; KCl, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KH_2PO_4, 1.0 g; $Ca(NO_3)_2 \cdot 4H_2O$, 2 to 6 g; $FeSO_4 \cdot 7H_2O$, 10 mg, plus 0.8 ml micronutrient solution as in Medium A. Medium C: Major components as in Medium B, plus sodium citrate, 1.5 g; no micronutrients.

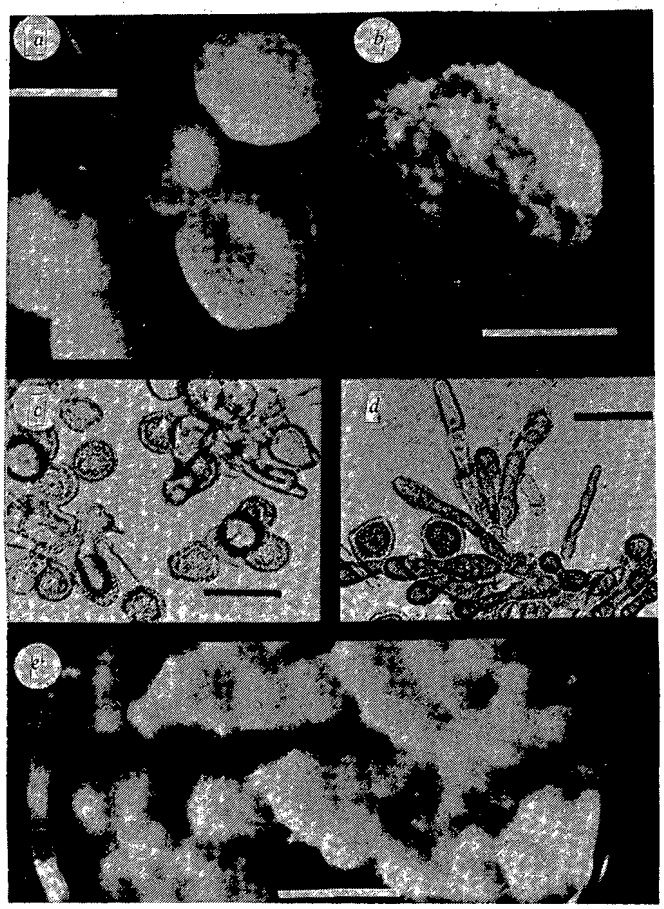

Fig. 1 Growth and development of *M. lini* (race 3) on liquid medium A (inorganic salts, sucrose, aspartic acid and cysteine). *a*, Fully developed vegetative colonies (1 month old) on liquid surface (scale bar, 0.5 cm); *b*, sporulating colony, 6 week old. The entire colony has developed into a uredosorus (scale bar, 0.5 cm); *c*, Uredospores (oblong cells) from colony like that in *b* (scale bar, 50 μm); *d*, teliospores from 2 month old colony (scale bar 50 μm); *e*, 1 month old vegetative colonies of *P. graminis* (race 15B-4) on liquid medium C (inorganic salts, glucose, glutamine and cysteine) (scale bar, 1 cm).

acid plus cysteine (2.3 or 4.6 mM). Sixty to seventy vegetative colonies per flask developed in 90–100% of the flasks within 4 weeks (primary axenic colonies). Two weeks later typical orange uredopustules were formed on the surfaces of most colonies (Fig. 1*a* and *b*) and dark brown teliosori appeared on some of them after 8 weeks growth. The uredospores and teliospores and their supporting hyphae were apparently closely similar to those found in nature (Fig. 1*c* and *d*). When uredospores produced *in vitro* were used to inoculate intact week-old flax cotyledons, infection and development of sporulating pustules occurred in a normal manner. When seeded on fresh medium, flax rust uredospores developed *in vitro* grew into secondary axenic colonies. These developed and sporulated in a manner indistinguishable from the primary axenic colonies. Uredospores from the secondary colonies also reinfected intact flax cotyledons. Decreases in the levels of aspartic or glutamic acid (22.5 mM) and cysteine (2.3 mM) resulted in poor growth and a low frequency of initiation of colonies, which failed to sporulate; glutathione was not essential for growth or sporulation. Replacement of aspartic acid with a high level of glutamine (42 mM) gave a high rate of colony initiation and excellent vegetative growth. These colonies were white and fluffy and had a yellowish stroma on the under surface, but did not sporulate and finally died.

An Australian isolate of *P. graminis tritici*, race 126-ANZ 6,7, developed large colonies on medium B plus high levels of aspartic acid (45 mM), cysteine (4.6 mM) and glutathione (3.3 mM); small areas of uredospores and teliospores developed on a few colonies in about 50% of the inoculated flasks. When uredospores formed by these colonies were placed on exposed mesophyll tissue they produced typical infections of seedling leaves of wheat[14]. Identical results were obtained on medium C plus 45 mM aspartic acid and 4.6 mM cysteine, so that glutathione and micronutrients were evidently not essential for growth. Of the three races of wheat stem rust, 126-ANZ 6,7 therefore most closely resembles race 3 of *M. lini* in its requirement for amino acids. Two North American isolates, races 15B-4 and 56, behaved in an entirely different manner and completely failed to grow on these media (Table 1).

Races 126-ANZ 6,7, 15B-4 and 56 all formed large vegetative colonies on medium C plus high levels of glutamine (42 mM) and cysteine (4.6 mM), but no sporulation occurred. Races 15B-4 and 56 also formed luxuriant vegetative colonies but did not sporulate on medium C plus aspartic acid, glutamine and cysteine (Fig. 1*e* and

Table 1). When glutamine was replaced with aspartic acid after 3 weeks' growth, vegetative growth continued and the stromata of individual colonies merged with each other, but there was no sporulation. Apparently aspartic acid will support vegetative growth of the wheat rust once it has started, but will not serve as a source of amino nitrogen for the initiation of colonies.

These results show that chemically-defined media can support luxuriant growth of wheat rust and both growth and sporulation of flax rust without the addition of complex organic substances like peptone or yeast extract. Further work will be required to discover the key to control of sporulation by the wheat rust fungus.

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Anti-receptor antibody and resistance to graft-*versus*-host disease

GRAFT-*versus*-host (GvH) disease is a complex syndrome initiated by the reaction of donor lymphoid cells against histocompatibility antigens of the host. Although newborn hybrid animals injected with parental-strain lymphoid cells develop fatal GvH disease, adult hybrid animals receiving weight-adjusted doses of such cells develop no clinically evident disease[1,2]. This resistance to GvH disease is age-dependent and radiosensitive; resistance develops at 3–4 weeks of age and is abolished by sublethal total-body irradiation[1]. Immunologically specific suppression of GvH reactions has been achieved by immunisation of F_1 hybrid host to produce antibody reactive with receptors on donor lymphoid cells for host antigens[3,4].

The GvH reactivity of lymphoid cells from the appropriate parental strain was suppressed in F_1 hybrids producing such anti-receptor antibody (ARA)[3,4]. This finding led to the suggestion that ARA could suppress GvH reactivity by blocking receptors on aggressor lymphocytes. We have investigated whether the natural resistance of adult F_1 hybrids to GvH disease is associated with the production of ARA. We found ARA in rats not developing the disease, while those rendered susceptible to GvH by irradiation did not produce ARA.

The radiosensitivity of the processes responsible for the resistance of adult rats to fatal GvH disease was confirmed as follows: groups of five adult female Lewis × Brown Norway (LBN) F_1 hybrid rats each either received 400 rad of total-body X irradiation or were left untreated. Twenty-four hours later each group received from 2.7×10^6 to 2×10^8 normal adult female Lewis spleen cells intravenously. Animals were weighed three times weekly and deaths were recorded daily (Fig. 1). All non-irradiated animals receiving Lewis spleen cells survived; none lost weight or had clinically evident disease. All irradiated hybrid rats, however, receiving more than 10^7 Lewis spleen cells died. Weight loss of up to 30% initial body weight preceded death. Control animals receiving 400 rad only had no clinical ill effects. Thus, non-irradiated LBN rats were resistant to fatal GvH disease when injected with 100 times more Lewis cells than were required to induce significant and often fatal GvH disease in those LBN rats pretreated with 400 rad of X irradiation.

Similar results were obtained when spleen cells from the Brown Norway parental strain were injected in analogous experiments. All unirradiated LBN rats receiving BN spleen cells in doses as high as 2×10^8 survived. All irradiated rats receiving more than 1×10^7 BN spleen cells died of GvH disease.

Sera from irradiated and nonirradiated adult hybrid rats receiving parental-strain spleen cells were assayed for ARA by two methods. One method involves a complicated bioassay in which ARA inhibits the formation of a chemotactic factor (product of antigen recognition or PAR) generated when parental-strain lymphoid cells are cultured briefly with hybrid lymphoid cells[5]. The other method involves measurement of the interaction between the specific alloantibody used for immunisation and serum from the immunised hybrid animal[3,6]. Such interaction can be detected by precipitin reactions in gel[3]. In a more sensitive assay, anti-receptor antibody can be detected and quantified by agglutination of sheep erythrocytes to which specific alloantibody has been coupled. ARA produced by immunisation of hybrid rats with alloantiserum and ARA produced by immunisation with parental-strain spleen cells have similar reactivity in all these assays.

To determine whether production of ARA correlated with the resistance of LBN animals to GvH disease, samples of serum were obtained from adult female LBN rats that had been injected with normal Lewis spleen cells. Groups of five LBN F_1 hybrid rats were given 400 rad of total-body X irradiation or were left untreated. Twenty-four hours later, 5×10^7 or 1×10^7 Lewis spleen

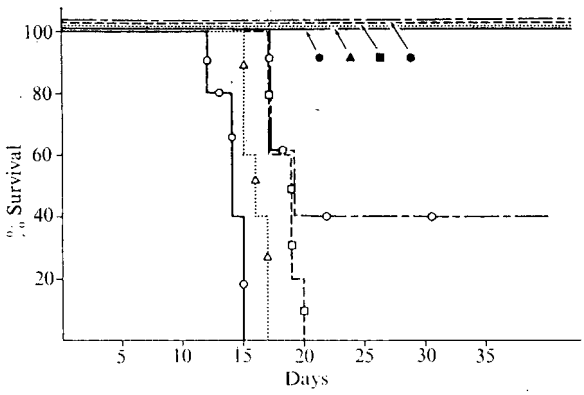

Fig. 1 Survival of adult female LBN F_1 rats after intravenous injection of normal adult female Lewis spleen cells. Groups of 5 F_1 rats each either received 400 rad of total body × radiation (open symbols) or were not irradiated (closed symbols). Twenty-four hours later, the rats were injected intravenously with either 2×10^8 (○ or ●), 5×10^7 (△ or ▲), 1.2×10^7 (□ or ■) or 2.8×10^6 (open and solid hexagons) normal Lewis spleen cells. No F_1 rats receiving 400 rad only (not shown) died.

Table 1 Development of ARA in F_1 hybrid rats receiving parental strain normal Lewis spleen cells

	ARA titre* Days after parental strain spleen cells					
	7	14	21	28†	35	42
5×10^7 Lewis spleen cells	0	0	0	64	4	0
	0	0	32	512	128	64
	0	0	16	256	32	0
	0	0	32	64	0	0
	0	0	8	16	0	0
1×10^7 Lewis spleen cells	0	0	128	256	Not done	Not done
	0	0	32	64		
	0	0	0	0		
	0	0	8	4		
	0	0	256	64		
400 rad plus 5×10^7 Lewis spleen cells	0	0	Dead			
	0	0	Dead			
	0	0	0	Dead		
	0	0	Dead			
	0	0	Dead			
400 rad plus 1×10^7 Lewis spleen cells	0	0	0	Dead		
	0	0	Dead			
	0	0	0	Dead		
	0	0	0	0	Dead	
	0	0	0	Dead		

* Reciprocal of the highest serum dilution which causes agglutination of sheep erythrocytes coated with the IgG fraction of Lewis anti-BN serum.

† These sera failed to agglutinate SRBCs coated with IgG fractions of Lewis anti-DA serum, Fischer anti-BN serum or BN anti-Lewis serum.

cells were injected intravenously and serum samples were obtained at weekly intervals. A haemagglutinin assay, involving sheep erythrocytes to which IgG antibody was coupled, was used to measure anti-receptor antibody. Lewis anti-BN antibody was purified by ammonium sulphate precipitation and elution from diethylaminoethyl-cellulose with 0.04 M phosphate buffer, *p*H 8.0. After dialysis against saline, the IgG antibody was coupled to sheep erythrocytes by means of chromic chloride[7]. Serial dilutions of serum were incubated with sensitised erythrocytes for at least 3 h and the settling pattern was observed (Table 1). Serum samples were also titered with normal sheep erythrocytes, and with sheep erythrocytes to which normal Lewis IgG, Lewis anti-DA IgG, or BN anti-Lewis IgG had been coupled.

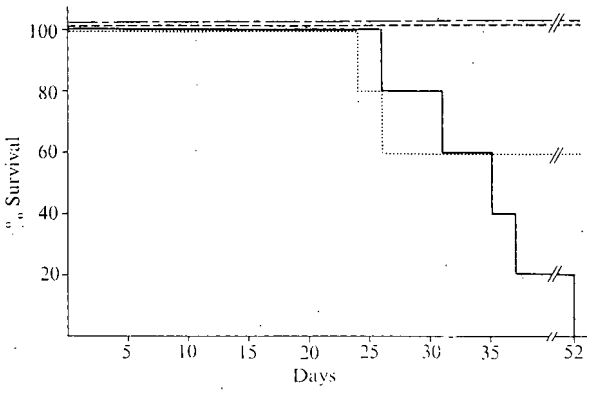

Fig. 2 Protection of irradiated LBN F_1 rats from lethal GvH disease by adaptive transfer of F_1 spleen cells. Irradiated LBN rats receiving 1×10^7 normal Lewis spleen cells only (———) all died of GvH disease. Irradiated LBN rats receiving 1×10^7 normal Lewis spleen cells and 5×10^7 spleen cells from F_1 rats resistant to GvH disease (— – —) suffered no mortality or weight loss. The group of irradiated LBN rats receiving 1×10^7 normal Lewis spleen cells and 5×10^7 normal LBN spleen cells (– – – – – –) had an intermediate mortality. LBN rats receiving 400 rad of X radiation only (—— ——) all survived. No additional mortality occurred in any group during the interval of 52–120 d.

As observed previously, all irradiated hybrid rats receiving 5×10^7 Lewis spleen cells and four or five irradiated rats receiving 1×10^7 Lewis spleen cells were dead within four weeks after cell injection. ARA could not be detected in any serum sample from the irradiated rats. None of the nonirradiated hybrid rats receiving Lewis spleen cells developed GvH disease. By 4 weeks after injection, 9 of 10 nonirradiated hybrid rats receiving Lewis spleen cells had measurable serum ARA. This antibody activity was first noted 3 weeks after injection and in most animals was no longer detectable 3 weeks later. The specificity of the ARA reactivity is indicated by the failure of serum samples obtained at 28 d to agglutinate erythrocytes coated with Lewis anti-DA IgG or with BN anti-Lewis IgG. The following observations indicate that ARA detected in the serum of hybrid rats receiving parental-strain lymphoid cells is an anti-idiotypic antibody and not a soluble complex of anti-BN antibody and BN antigen formed in antigen excess. Such a complex would be expected to agglutinate sheep erythrocytes coated with anti-BN antibody of any idiotype, whereas anti-idiotypic antibody would agglutinate only erythrocytes coated with anti-BN antibody of the appropriate idiotype. Fischer anti-BN and Lewis anti-BN IgG fractions possessing equivalent titres of anti-BN haemagglutinin antibody were used to coat sheep erythrocytes. The two preparations of coated erythrocytes agglutinated to the same extent with a rabbit anti-rat immunoglobulin serum. Serum from hybrid rats obtained 28 d after injection of Lewis spleen cells agglutinated Lewis anti-BN coated cells (Table 1), but did not agglutinate erythrocytes coated with Fischer anti-BN antibody. This pattern of agglutination indicates the anti-idiotypic nature of ARA.

Thus, ARA is detected in the serum of hybrid rats that do not develop GvH disease. Irradiation renders hybrid rats susceptible to fatal GvH disease; irradiated rats do not produce detectable amounts of ARA. Production of ARA may be a part of the radiosensitive process by which hybrid animals resist fatal GvH disease. If ARA plays such a protective role, lymphoid cells from hybrid rats producing ARA should enable irradiated hybrids to resist fatal GvH disease. To test this hypothesis, we gave LBN rats 400 rad of total-body X radiation. Twenty-four hours later, they received one of the following: 10^7 Lewis spleen cells; 10^7 Lewis spleen cells plus 5×10^7 normal LBN spleen cells, or 10^7 Lewis spleen cells plus 5×10^7 spleen cells from LBN rats that had previously received two injections of Lewis spleen cells. All the previously injected LBN rats had ARA in their serum when their spleen cells were taken. Irradiated LBN rats given spleen cells from the LBN rats that had resisted fatal GvH disease were protected from the lethal effects of injection of parental spleen cells (Fig. 2). Normal LBN spleen cells were somewhat less effective in conferring protection to irradiated hybrid rats.

Thymus-derived (T) lymphocytes must be present in the responder population[8] to produce GvH reactions. Recent evidence obtained from GvH reactions *in vivo*[9] and mixed lymphocyte cultures *in vitro*[10] indicates that genetic disparity at one or more lymphocyte-defined (LD) loci closely linked to the serologically defined (SD) major histocompatibility complex is essential for a strong proliferative response. The generation of cytotoxic effector cells appears to require in addition a disparity at one or more of the SD loci. Cyto-

toxic effector cells, like the cells responsive to the LD loci, are believed to be T lymphocytes[11].

The generation of effector cells in culture requires DNA synthesis[12]. Cultures treated so as to eliminate cells that have undergone DNA synthesis do not yield cytotoxic effector cells[13]. Since treatment to eliminate cells that had undergone DNA synthesis could be delayed until placing the cells into the effector cell assay, the cytotoxic effector cells must have replicated during the sensitisation process. Thus it seems that proliferation of both LD responsive and SD responsive lymphocytes is required for full cytotoxic expression.

Several groups have suggested a model of cellular cooperation involving two populations of T lymphocytes to explain the apparent requirement for both LD and SD responsive T cells. Hayry and Andersson[14] suggest that the effector cell responsive to SD antigen requires cooperative interactions of an LD responsive T lymphocyte (and perhaps an adherent cell as well) before it can divide and cause significant target cell lysis.

Local GvH reactions may involve predominantly the recognition and proliferative phases of the immune response. The syndrome of GvH disease, however, probably includes an additional component of cell-mediated injury initiated by effector lymphocytes[8]. If such effector lymphocytes react with the SD antigens of the host through an immunologically specific receptor molecule, then blockade of that receptor may lead to a blunting or inhibition of the killer cell potential of these lymphocytes. One immunologically specific receptor molecule able selectively to bind SD antigen is antibody. We suggest that the anti-idiotypic antibody demonstrable in the serum of F_1 hybrid rats after injection of parental strain cells may function by blocking or eliminating those parental lymphoid cells which display receptors for the serologically defined histocompatibility antigens of the host.

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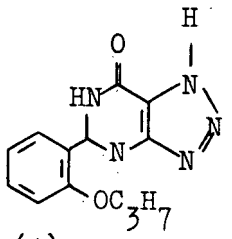
New inhibitor of reagin-mediated anaphylaxis

Methylxanthines, such as theophylline and caffeine, inhibit the antigen-induced release of histamine and of slow reacting substance of anaphylaxis (SRS-A) from passively sensitised human lung and human basophilic leukocytes[1,2]. We have shown that these methylxanthines also inhibit a passive cutaneous anaphylactic (PCA) reaction mediated by reaginic antibodies in the rat. The methylxanthines are, however, from 20 to 50 times less potent than disodium cromoglycate. Because of the relatively low potency of theophylline and because of its other pharmacological actions, notably as a bronchodilator, it is not clear to what extent inhibition of reagin-mediated anaphylaxis may be important in its use for the treatment of allergic bronchial asthma.

By progressive modification of the chemical structure of methylxanthines, we have found that certain 2-phenyl-8-azapurin-6-ones[3] possess high and selective anti-allergic activity. In particular, 2-*o*-propoxyphenyl-8-azapurin-6-one, M&B 22,948 (1), is from 20 to 50 times more potent than disodium cromoglycate in inhibiting reagin-mediated anaphylaxis in a number of test systems and is also active by oral administration.



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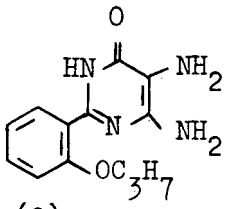

(2)

2-*o*-Propoxyphenyl-8-azapurin-6-one is a white crystalline solid, melting point 241° C (decomp.). It is less than 0.1% w/v soluble in water but readily forms salts, for example the triethanolamine salt has a melting point of 138–139° C. The azapurinone was prepared by nitrous acid treatment of the diamine (2) which itself was prepared from *o*-propoxybenzamidine by conventional methods.

M&B 22,948 has been compared with disodium cromoglycate in three experimental models involving reagin-mediated anaphylaxis. These were first, the inhibition of the allergen induced release of histamine and SRS-A from passively sensitised human lung *in vitro*[4], second, the inhibition of a reagin-mediated PCA reaction in the rat[5] and third, the inhibition of reagin-mediated anaphylactic bronchospasm in the guinea pig. In experiments involving aqueous solutions, M&B 22,948 was used as its triethanolamine salt. Doses refer to the parent azapurinone.

M&B 22,948 was more potent than disodium cromoglycate in inhibiting the anaphylactic release of both histamine and SRS-A from human lung tissue (Fig. 1). The effective concentration range for each drug was fairly narrow. The optimum concentration for inhibition of mediator release determined in at least seven separate experiments was in the range of 1–3 μg ml^{-1} for M&B 22,948 and 30–100 μg ml^{-1} for disodium cromoglycate.

The intravenous dose of M&B 22,948 causing 100% inhibition of the rat PCA reaction was from 35–50 times less than that of disodium cromoglycate. Thus, complete inhibition of the rat PCA reaction was consistently obtained with intravenous doses

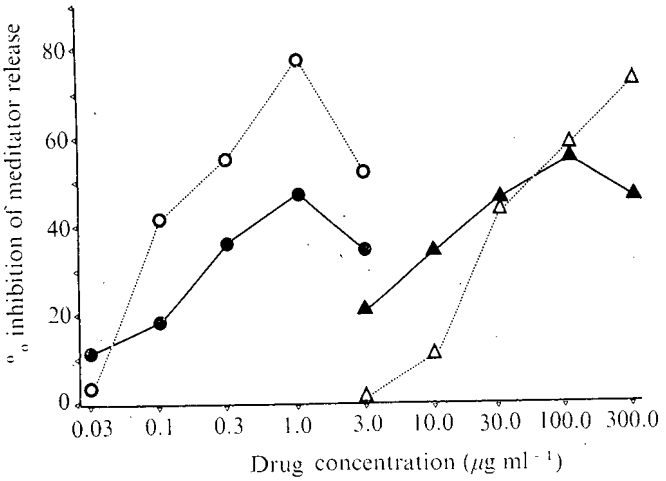

Fig. 1 Inhibition of allergen-induced release of histamine and SRS-A from passively sensitised human lung tissue by M&B 22,948 and disodium cromoglycate in two separate experiments. Using M&B 22,948: ●, histamine; ○, SRS-A. Using disodium cromoglycate: ▲, histamine; △, SRS-A. Chopped human lung was incubated for 18 h with serum from asthmatic subjects. Aliquots were challenged with extracts of *Dermatophagoides farinae* (500 μg ml^{-1}) immediately after the addition of graded concentrations of the test compounds. Histamine was assayed fluorometrically. SRS-A was assayed on the mepyraminised guinea pig ileum. Points refer to single determinations from three pooled samples at each drug concentration.

of 0.1 mg kg^{-1} of M&B 22,948 and 2–5 mg kg^{-1} of disodium cromoglycate. Intravenous theophylline had approximately one-fiftieth the potency of disodium cromoglycate.

When administered orally to rats 15 min before allergen challenge, M&B 22,948 was effective in inhibiting the PCA reaction. The dose response curve was 'bell shaped' (Fig. 2) and complete inhibition of the PCA reaction was rarely obtained. Maximal inhibition was obtained with oral doses of between 0.5–2.0 mg kg^{-1}. Disodium cromoglycate showed poor to negligible activity; an oral dose of 500 mg kg^{-1} produced less than 30% inhibition when administered either 15 or 40 min before allergen challenge. Theophylline showed some oral activity with 80% inhibition of the PCA reaction occurring with a dose of 100 mg kg^{-1}.

Anaphylactic bronchospasm was produced in the guinea pig passively, systemically sensitised with *Trichinella spiralis* antiserum and premedicated with propranolol (5 mg kg^{-1}, intraperitoneally). The mean preconvulsion time of groups of a minimum of six sensitised guinea pigs exposed to an aerosol of allergen was increased by 19%, 50% and 69% following intravenous doses of 5, 10 and 20 mg kg^{-1} of M&B 22,948 (Table 1). Disodium cromoglycate (100 mg kg^{-1} intravenously) produced a protection comparable to that of 10 mg kg^{-1} of intravenous M&B 22,948. An oral dose of 200 mg kg^{-1} M&B 22,948 produced a significant protective action. Maximum protection was afforded when the animals were challenged 15 min after receiving 200 mg kg^{-1} M&B 22,948 orally but a significant increase in the preconvulsion time was also seen when challenge was carried out 4 h after drug administration (Table 1). No reduction in the severity of anaphylactic bronchospasm was produced with oral doses of up to 200 mg kg^{-1} of disodium cromoglycate, administered 15 or 40 min before antigen challenge. Theophylline has not been studied in these tests.

M&B 22,948 antagonised some of the pharmacological effects of the mediators of anaphylaxis. Thus, concentrations of M&B 22,948 of 1–30 μg ml^{-1}, which inhibit the anaphylactic release of mediators from human lung tissue, also antagonised the spasmogenic effects of histamine, SRS-A and $PGF_{2\alpha}$ on isolated human bronchial muscle. In the rat, intravenous doses of 10–25 mg kg^{-1} of M&B 22,948 were required to reduce the increase in capillary permeability produced by intradermal histamine and 5-HT. These dose levels are considerably higher than that of M&B 22,948 required to inhibit the reagin-mediated PCA reaction (0.1 mg kg^{-1} intravenously).

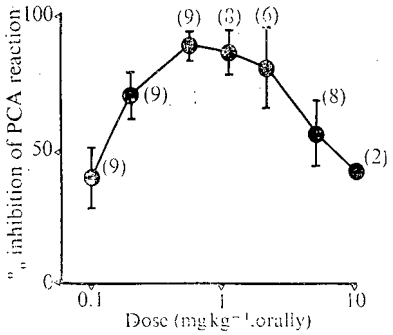

Fig. 2 Inhibition by oral M&B 22,948 of reagin-mediated PCA reactions in the rat. Rats were sensitised by intradermal injection of *Nippostrongylus brasiliensis* antiserum. 48 h later they were challenged intravenously with *N. brasiliensis* extract. M&B 22,948 was administered orally 15 min before challenge. Each point is the mean percentage inhibition of the PCA reaction determined in the number of animals indicated in parentheses ± standard error.

Table 1 Inhibition of reagin-mediated anaphylactic bronchospasm in the guinea pig by M&B 22,948 administered intravenously, orally or by the inhalation of an aqueous aerosol

Route of drug administration	Dose (mg kg^{-1})	Time (min) between dose and bronchial allergen challenge	Mean % protection
	5	1	19 (4)†
Intravenous	10	1	50 (4)
	20	1	69 (4)
		15	62 (4)
		40	54 (2)
Oral	200	60	48 (6)
		120	43 (3)
		240	42 (3)
Inhalation	50 (mg ml^{-1})	1	34(11)

The % protection was 100 $[1-(C/T)]$ where C and T were the preconvulsion times of control and treated groups.

*Concentration of aerosol generating solution. Animals exposed to aerosol for 2 min.

†Figures in parentheses refer to the number of experiments.

The concentrations of M&B 22,948 (μg ml^{-1}) required to cause a 50% reduction in the heights of the contractions of superfused guinea pig ileum produced by various agonists were as follows: histamine, 32; SRS-A, 7; acetylcholine, 1; PGE_2, 15; $PGF_{2\alpha}$, 2.5; 5-HT, 6.6. All these values were within the range of concentrations which inhibit the reagin-mediated anaphylactic release of histamine and SRS-A from human lung tissue. As the histamine released from the human lung tissue was determined fluorimetrically, the antihistamine activity of M&B 22,948 would not have interfered in the assays. M&B 22,948 showed no significant activity, either intravenously or orally, in protecting conscious guinea pigs from histamine-induced bronchospasm. Similarly, in experiments carried out in the anaesthetised guinea pig, using either the Konzett–Rössler or the Dixon–Brodie method of recording bronchial tone, no indication was obtained of bronchodilator action or of a specific antihistamine or anti-5-HT action of M&B 22,948.

Certain 2-phenyl-8-azapurin-6-ones, and in particular M&B 22,948, have been shown to have high activity in inhibiting the release of the pharmacological mediators of anaphylaxis from isolated human lung tissue *in vitro*, in a reagin-mediated PCA reaction in the rat, and in reagin-mediated anaphylactic broncho-

spasm in the guinea pig. The potency of M&B 22,948 was up to 50 times that of disodium cromoglycate, depending on the test system and the method of drug administration. M&B 22,948 may be of interest in the prophylactic treatment of allergic bronchial asthma. Since this work was carried out, we have learnt of the oral activity of an oxoxanthene derivative (AH 7725) in inhibiting an allergen induced immediate-type asthmatic response in man[6].

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Significance of immunofluorescent staining of lymphocytes with antisera to IgM immunoglobulins

IMMUNOLOGICAL reactions depend for their completeness on at least two different populations of lymphocytes, one derived from the bone marrow and the other from the thymus (B and T cells). B lymphocytes, precursors of the antibody-forming cells of vertebrates, are recognised *in vitro* by two characteristic reactions: surface fluorescence with antisera to immunoglobulins[1] and rosette formation with red cells sensitised with some components of complement[2]. Since the cells that show surface immunofluorescence are retained on columns of beads coated with antibodies to immunoglobulins[3], surface fluorescence is generally interpreted to mean that immunoglobulins are present on the surface of B lymphocytes[4].

Various means have been devised to prove this point primarily by using high resolution optical devices[5] or specific antisera[6]. Although antisera to IgM immunoglobulins bind specifically to structures on the surface of lymphocytes, in only two cases to our knowledge have IgM immunoglobulins been separated from lymphocytes[7,8] and in such small quantities that the authors had to rely on measurements of radioactivity of subcellular fragments obtained by specific coprecipitations to support their assertions. It is generally believed as a result of these and other experiments, that lymphocytes indeed bear immunoglobulins and in particular IgM immunoglubulin, on their surface. We were therefore surprised to find that it was possible to

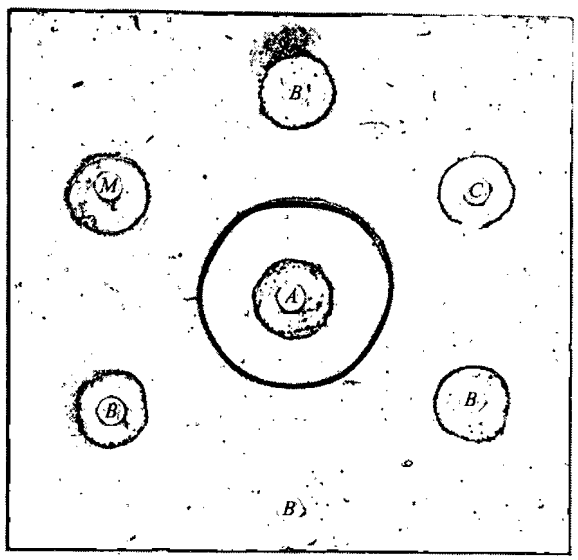

Fig. 1 Double diffusion in agar of IgM immunoglobulin (*A*) with anti-CHO (*C*), anti-μ (*M*), and anti-IgM (*B*). The reaction shows complete immunological identity of the three antisera with IgM immunoglobulin. Purified IgM immunoglobulin was prepared by gel filtration of 1.4 l of normal human serum on columns of Biogel P300 resin. The fraction eluting in the exclusion volume of the resin was concentrated over Ficoll and applied to a column of ethanolysed cellulose in a Tris-borate buffer of *p*H 9.4. The colloids were electrophoresed at a potential of 240 V for 20 h at 4° C and eluted from the column at a rate of 10 ml h^{-1}. The fractions reacting with an antiserum to human IgM immunoglobulin were pooled, dialysed against 0.15 M NaCl, and re-electrophoresed. α-macroglobulin could be separated from the IgM in this way. Fractions containing IgM immunoglobulin were chromatographed twice on Sephadex G200 resin to a single homogeneous chromatographic peak (0.330 g). By electrophoresis, sedimentation and precipitation with an antiserum to human IgM immunoglobulin over 98% of the protein contained in this fraction was shown to be IgM immunoglobulin. At no time during this preparation were the solutes containing IgM immunoglobulin frozen. The purified IgM was digested first with trypsin (1% w/w) at *p*H 8.0 in 0.65 M Tris HCl containing 0.01 M $CaCl_2$ for 24 h at 37° C, followed by pronase (1% w/w) under the same conditions for an additional 24 h at 37° C. The digested protein was dialysed against distilled water. The dialysate was concentrated *in vacuo* and applied to a column of Sephadex G25 (fine) resin (0.8 × 200 cm) in 1% ammonium bicarbonate. Materials eluting from the Sephadex resin were analysed for carbohydrate by the Mohlish reaction, and the Mohlish-positive materials were rechromatographed on Sephadex G25 to a single symmetrical peak. The materials within this peak were concentrated *in vacuo* and separated by paper chromatography (butanol–ethanol–acetic acid–water 8:2:1:3). Five oligosaccharides were separated in this way. The anti-CHO antiserum (*C* above) is specific for these five oligopeptides.

quantify IgG separable from human B cells[9] and that human B lymphocytes could be induced to synthesise IgG *in vitro*[10]; but we were unable either to quantify IgM or to show synthesis of IgM in these cells even though they fluoresced with antisera to IgM immunoglobulin. Some infants with severe combined immunodeficiency possess in their serum a monoclonal IgG; we have shown that lymphocytes from the serum of one of these children could be induced to synthesise *in vitro* the monoclonal IgG that was in the child's serum[11]. Yet all of his lymphocytes showed surface fluorescence with an antiserum to IgM. We decided to study this apparent discrepancy. We wish to present evidence which will show that surface immunofluorescence of lymphocytes with antisera to IgM is not caused by the presence of IgM on the surface of these lymphocytes.

Human IgM was prepared by conventional physicochemical procedures from normal human serum. Over

94% of the protein in this preparation sedimented in the centrifuge as a single boundary with a sedimentation coefficient of 19S. It precipitated quantitatively with an antiserum to human IgM.

Proteolysis of IgM with trypsin and pronase has been shown to degrade extensively both μ and L chains of IgM[12]. Five glycopeptides containing a minimum of 5 and a maximum of 13 sugar residues each, together with six to nine amino acids, represent the only material separable in the exclusion volume of a Sephadex G25 resin when a trypsin-pronase digest of IgM is analysed. Each oligopeptide contains N-acetyl glucosamine as the reducing carbohydrate end group, and a fucose (oligopeptide 1 and 2) or a mannose (oligopeptide 3 to 5) in each case bound to an aspartic acid of the polypeptide chain, representing the non-reducing carbohydrate ends. These five glycopeptides comprise all the carbohydrate of the μ chain[13]. Purified IgM was digested with trypsin followed by pronase. The glycopeptides obtained after hydrolysis were separated on Sephadex into Mohlish-positive materials that on paper chromatography (butanol–ethanol–acetic acid–water 8:2:1:3) were shown to be composed of five chemical entities each containing carbohydrate residues. We obtained 10 mg of these glycopeptides (dry weight).

An immunoadsorbent resin was prepared by a modification of the method of Axen and Ernback[14]. To assure that the carbohydrate moiety of the glycopeptides would bind to the immunoadsorbent, Sepharose 4B activated with CNBr was coupled to L-lysine. CNBr-activated IgM glycopeptides were in turn, coupled to the Sepharose–lysine adduct. Over 84% of the carbohydrate was bound to the Sepharose in these conditions.

IgG globulin was separated (DEAE cellulose) from a rabbit antiserum to normal human IgM globulins. The antiserum was rendered monospecific for the μ chains of IgM by passage through immunoadsorbents made by coupling human IgG and IgA immunoglobulins and BenceJones proteins to Sepharose 4B. This rabbit IgG globulin (1 g) was incubated at 37° C for 2 h with the immunoabsorbent containing the glycopeptides of IgM in phosphate-buffered saline (PBS) at *p*H 7.2. The unbound globulin was eluted at room temperature, and the absorbent washed with PBS. Bound antibodies were eluted with a glycine/HCl buffer, *p*H 2.0 (0.2 M). After five adsorption cycles, no antibodies remained bound to the immunoadsorbent. A total of 3% of the rabbit IgG fraction was eluted as specific antibody to the glycopeptides (anti-CHO). The remainder was designated anti-μ even though antibodies to μ determinants of IgM were not specifically isolated. Both fractions (anti-CHO and anti-μ) reacted with human IgM as shown in Fig. 1. Both antisera gave reactions of complete fusion against IgM. Immunodiffusion in agarose (1.5%) rather than agar did not alter the pattern of precipitation. Neither the anti-CHO nor the anti-μ precipitated in agar with the glycopeptides isolated from IgM as described above, showing that the glycopeptide fraction did not contain large μ-chain fragments that could react with either the fractionated or non-fractionated antiserum. The anti-CHO and anti-μ antibodies had sedimentation coefficients of 6.8 and 6.6, respectively, indicating that neither antibody contained aggregated γ globulin. Fluorescent antisera were prepared by dialysing the proteins against fluorescein isothiocyanate buffered at *p*H 9.0 (ref. 15). Fluorescein-labelled anti-IgM, anti-CHO, and anti-μ were reacted with B cells. The results are summarised in Table 1.

B cells were separated from peripheral blood lymphocytes or from tonsillar lymphocytes by density centrifugation on gradients of bovine serum albumin[16]. Two different populations of B cells were obtained from the gradients, both of which possessed a complement receptor indicated by rosette formation of these cells with EAC1423. One cell population comprised large cells that sedimented in the upper region of the gradient (17–21% albumin, B1). These could not be induced to form IgG *in vitro*. The second cell population comprised small cells with a high density which sedimented toward the bottom of the gradient (29–31% albumin, B2), and these could be induced to form IgG immunoglobulins *in vitro*. Over 95% of B1 cells reacted with fluorescein-labelled anti-IgM or anti-CHO, but only 55% of B2 cells did so. Less than 2% of either B1 or B2 cells reacted with anti-μ. For comparison, less than 2% of B1 and 20% of B2 cells reacted with fluoresceinated anti-IgG. Upwards of 80% of the fluorescence of B1 cells and 70% of B2 could be inhibited when the cells were preincubated with unlabelled anti-CHO or anti-IgM, but the fluorescence remained unaltered when either cell was preincubated with anti-μ. The specificity of the antisera was tested by incubating the bone marrow of a patient with Waldenström macroglobulinemia with fluorescein-labelled anti-IgM, anti-μ and anti-CHO.

Two types of fluorescent pattern were visible; cytoplasmic fluorescence and surface fluorescence. The cytoplasmic fluorescence of the plasmacytoid cells of the marrow synthesising IgM was revealed both with the anti-IgM and anti-μ antisera. This experiment (positive control for anti-μ) indicated that the anti-μ antiserum had activity against the μ chain determinants of IgM. This activity could be inhibited by preincubating the antiserum with IgM but not with the glycopeptides of IgM, showing specificity of this antiserum for the polypeptide chains of IgM. A small amount of B1 cells were present in the marrow (10%), and presumably it was these cells that reacted with the antiserum to IgM or to the glycopeptides in a pattern showing surface fluorescence. This activity could be inhibited by preincubating the antiserum with the glycopeptides but not with IgM showing specificity of the antiserum for the glycopeptides of IgM but not for the polypeptide chains of IgM. Comparable results were obtained when the following experiments were done. Anti-IgM antiserum (5 ml) was adsorbed 10 times with 10^7 B1 cells each time at 37° C for 1 h. The adsorbed antiserum showed unimpaired reactivity with IgM by double diffusion in agar. It failed, however, when preincubated with either B1 or B2 cells to inhibit the fluorescence of the anti-CHO antiserum with both B1 and B2 cell populations.

Table 1 Percentage of cells staining with fluorescein-labelled antisera to human IgG and Igm Immunoglobulins and the glycopeptides (CHO) or polypeptide chains (μ) of IgM

Cell type	Pre-incubation of Cells with antisera to	Antisera with antigen	Fluorescein-labelled antibody (% of cells staining) IgM	CHO	μ	IgG
B1*			94(91–99)	95(91–99)	<2	<2
B1	CHO		20(12–32)	20(12–32)		
B1	IgM		22(10–33)	16(8–26)		
B1	μ		91(90–96)	94(90–98)		
B2†			55(45–70)	56(50–62)	<2	20
B2	CHO		19(12–27)	15(10–22)		
B2	IgM		17(9–23)	14(8–21)		
B2	μ		54(42–66)	56(46–69)		
BM‡			40§	10	40§	
BM		IgM	10	10	<2	
BM		CHO	40	<2	40	

Fluorescent antisera were prepared by dialysing the protein to be labelled (1 ml of a 1% solution) against 300 ml of a solution containing fluorescein isothiocyanate (0.5 mg ml^{-1}) buffered at *p*H 9.0 for 72 h in the dark. Labelled antisera were dialysed extensively against PBS and stored in the dark. 0.1 ml of fluorescein-conjugated antisera was added to an equal volume of a suspension containing 2×10^6 cells ml^{-1} in medium 199 supplemented with 5% foetal calf serum and 10^{-2} mol l^{-1} sodium azide. Cells were incubated for 45 min in the cold, washed three times in medium 199, and transferred to a glass slide in a drop of glycerol:PBS (9:1). Slides were examined, and the percentage of fluorescent cells recorded.

* Six determinations.

† Four determinations.

‡ Bone marrow from a patient with Waldenström's macroglobulinemia (one determination).

§ Cytoplasmic staining (all others were surface stained).

Immunoglobulins are glycoproteins. Over 10% of the mass of IgM is carbohydrate so that antisera to IgM should contain antibodies directed to the carbohydrate, as well as to the polypeptides of the immunoglobulin chains. The data presented here show that immunofluorescence of B lymphocytes with antisera to IgM is not caused by IgM immunoglobulin present on the surface of B lymphocytes. It is rather caused by interaction of antisera to IgM immunoglobulin with carbohydrate on the surface of the lymphocyte. This carbohydrate must be cross reactive and, therefore, structurally similar to the carbohydrate of IgM immunoglobulin.

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Cellular immune response to a drug-treated L5178Y lymphoma subline

TUMOUR-specific transplantation antigens (TSTA), have been detected on a wide range of experimental tumour cells and elicit weak immunogenic activity. As a rule, little immune response of the host to autocthonous tumours or to implanted syngeneic tumours has been detected. Furthermore, immunological techniques, such as active immunisation, passive transfer of antibodies or adoptive transfer of immune lymphocytes, have not been entirely satisfactory. Attempts have also been made to increase the immunogenic properties of tumour cells by *in vitro* enzymatic treatments[1] or *in vitro* coating of antigenic determinants on cancer cells[2].

Some studies have shown that it is possible to increase the immunogenicity of tumour cells[3]. In our laboratory immunogenic L1210 sublines were induced by prolonged *in vivo* treatment with the drug 5-(3,3-dimethyl-triazeno)imidazole-4 carboxamide (DIC)[4]. Mice compatible with the untreated L1210 leukaemia were resistant to a heavy challenge of L1210/DIC cells, whereas host resistance could be abrogated by immunodepression. The alteration of antigenicity acquired by L1210 DIC-treated cells (L1210/DIC) was found to be heritable[4,5].

We present evidence that a subline of L5178Y tumour, transformed by DIC *in vivo* (L5178Y/DIC) (manuscript in preparation) implanted into mice compatible with the parental leukaemia, stimulated a specific cell-mediated cytotoxic response.

L5178Y/DIC was maintained by serial intraperitoneal (i.p.) passages in immunosuppressed 200 mg kg^{-1} (cyclophosphamide given intraperitoneally 24 h before tumour challenge) hybrid CDF_1 mice. Other tumour lines used in this study were maintained by serial i.p. passages in compatible inbred mice.

The cellular immune response was evaluated by the *in vitro* ^{51}Cr release assay[6], modified by Canty[7].

A sample of 10^7 spleen cells (attackers) from normal (control) or immune mice were suspended in a plastic Petri dish (Falcon, 35 × 10 mm) in 1 ml Medium 199 (DIFCO) supplemented with 10% heat inactivated foetal calf serum (GIBCO) and incubated for 4 h with 5×10^4 (50 µl) ^{51}Cr-labelled cells (targets) on a rocking platform at 37° C. in a moist atmosphere of 10% CO_2–90% air. Radioactivity released in the supernatant by labelled target cells was counted in an automatic scintillation counter.

In each experiment quadruplicated samples were used for each experimental group and the data reported are from one out of three similar experiments. The percentage cytotoxicity for each group was calculated as follows:

$$\frac{\text{c.p.m. } ^{51}\text{Cr released from incubated cells}}{\text{c.p.m. } ^{51}\text{Cr released from frozen–thawed cells (4 times)}} \times 100$$

Experimental results were expressed as (% cytotoxicity in experimental samples)−(cytotoxicity in control samples). The ^{51}Cr release in control samples was 12 ± 3%.

Spleen cells from mice immune to L5178Y/DIC tumour cells were highly cytotoxic *in vitro* to ^{51}Cr-labelled L5178/DIC cells. Parental radiolabelled L5178Y cells and other tumour and thymus cells syngeneic or allogeneic with CDF_1 mice, did not release the isotope after incubation with anti-L5178Y/DIC attacker cells. The specific cell-mediated ^{51}Cr release indicates

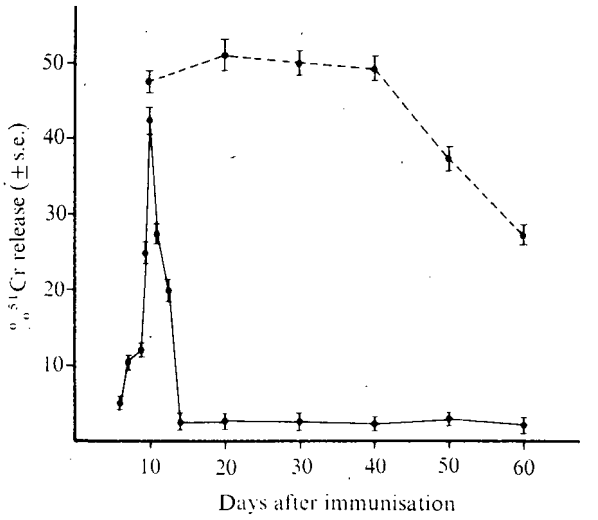

Fig. 1 Kinetics of immune spleen cell activity. —, Direct assay; ---, assay 24 h after incubation. Attacker cells were from CDF_1 mice immunised with 10^7 L5178Y/DIC viable cells injected i.p. and assayed against ^{51}Cr-labelled L5178Y/DIC cells. In these and the following assays the ratio of attacker cells to labelled target cells was 200:1.

Table 1 Cytotoxic activity of spleen cells immunised against allogeneic antigens

Attacker cells	Target cells	^{51}Cr release (±s.e.)
	EL_4	14.1 (0.4)
S_{EL_4}	L5178Y	3.4 (0.4)
	L5178Y/DIC	2.8 (0.6)
	$E\delta G_2$	16.6 (0.8)
$S_{E\delta G_2}$	L5178Y	3.4 (1)
	L5178Y/DIC	−2 (0.9)
	C_3H	42.8 (0.7)
S_{C_3H}	L5178Y	3.3 (0.9)
	L5178Y/DIC	−1.8 (0.9)
	C57BL10/Sc Cr	12.4 (1)
$S_{C57BL/10\ Sc\ Cr}$	L5178Y	2 (0.6)
	L5178Y/DIC	3.5 (0.8)

Attacker cells (S) were spleen cells from CFD_1 mice 10 d after immunisation with an i.p. inoculum of 10^7 tumour cells (EL_4–$E\delta G_2$) or a Sc implant of 1 mm³ skin tissue pieces (C_3H–C57BL/10 Sc Cr). L5178Y and L1210 were from Microbiological Ass. Bethesda. EL4 and E♂G2 were obtained from Dr. G. Della Porta, National Cancer Institution Milan. Moloney Sarcoma (MSV) and Gross leukaemia (GLV) were virus-induced by Dr A. Di Marco, National Cancer Institution, Milan.

that DIC treatment can induce transplantation antigen(s) on L5178Y/DIC cells, not detectable on the original L5178Y lymphoma and on other tumour, or normal thymus cells.

The cytolytic activity of spleen cells from CDF_1 mice assayed at different days after administration of the immunising inoculum is shown in Fig. 1. The activity reached a peak at 10 d after tumour cell inoculation and could not be detected after 13 d. 24 h *in vitro* incubation fully reactivated the cytotoxic activity and it was still evident at 60 days following immunisation. The immune cell reactivity, its disappearance a few days after the peak was reached as the result of a block by some still unknown factor and its reactivation by 24 h incubation, agree with previous observations involving the ^{51}Cr release assay technique[8].

Immune spleen cells from CDF_1 mice sensitised to allogeneic antigens were cytotoxic to ^{51}Cr-labelled tumour cells bearing the corresponding alloantigens (Table 1). The spleen cells failed to release the radiolabel from either the original L5178Y or the L5178Y/DIC suggesting that the cells of the subline had not acquired some nonspecific immunosensitivity. Evidently neither L5178Y or L5178Y/DIC associated antigens can react with *H*—2^b or *H*—2^k associated antigens.

The specificity of L5178Y/DIC antigen(s) was determined by using an assay for inhibition of cytotoxicity involving addition of unlabelled cells. Addition of unlabelled cells to the mixture of immune lymphocytes and labelled L5178Y/DIC target cells would result in inhibition of ^{51}Cr release if the unlabelled cells shared some common antigen(s) with L5178/DIC cells. Only unlabelled L5178Y/DIC cells elicited marked inhibitory activity (Table 2). All other normal or tumour cells added, including the parental L5178Y cells, did not modify ^{51}Cr release significantly. The failure to detect L5178Y/DIC antigens on foreign cells provides strong evidence of the specificity of L5178Y/DIC induced antigen(s).

Table 2 Inhibition of immune spleen cell-mediated cytotoxicity by addition of unlabelled cells

Added inhibitor cells	% Reduction in lysis*	
	5×10^5	10^5
L5178Y/DIC	68 (7.8/24.9)	39 (15.3/24.9)
L5178Y	6 (15.6/16.6)	−6 (17.6/16.6)
L1210	8 (22.9/24.9)	4 (24/24.9)
$E\delta G_2$	−2 (39/38)	−5 (40/38)
EL_4	13 (22.7/26.4)	13 (22.7/26.4)
MSV	12 (14.3/16.1)	4 (15.4/16.1)
GLV	16 (13.5/16.1)	0 (16.1/16.1)
Spleen DBA/2 Cr	−11 (21/18.8)	0 (18.8/18.8)
CDF_1	0 (18.8/18.8)	2 (19.1/18.8)
BALB/c	8 (23.5/25.5)	3 (24.7/25.5)
C57BL/10 ScCr	4 (18/18,8)	8 (17.2/18.8)
C_3H	8 (23.5/25.5)	4 (24.5/25.5)

Attacker cells were from CDF_1 mice i.p. challenged with 10^7 L5178Y/DIC viable cells and assayed after 10 d against ^{51}Cr labelled L5178Y/DIC cells.
* % lysis with inhibitor cells / % lysis without inhibitors.

Immune cells were assayed immediately or 4 h after X-ray treatments. The activity of the attacker cells was only partially reduced even by heavy doses of X rays (2.00–4.00 r.), in accordance with the known resistance of cytolytic cells (immune lymphocytes and macrophages) to X irradiation. (Table 3)[9].

Our studies indicate that L5178Y/DIC lymphoid cells carry new antigen(s) not detectable on parental cells. The mechanism, by which DIC alters the immunological properties of L1210 and L5178Y lymphomas is unknown. The drug-induced antigen(s) were not found on normal lymphocytes or on cancer cells. But, the strong transplantation antigen(s) seems to be a permanent genetic feature, as the L5178Y/DIC cells maintained the acquired antigen(s) even when the drug treatment was discontinued over a long period.

Table 3 Cytotoxic activity of X-irradiated immunised spleen cells

X-ray dose	% lysis (± s.e.)	% Reduction in lysis relative to non-irradiated cells
Immediate assay		
—	20.3 (0.9)	—
500 r.	19.4 (0.8)	5
1,000 r.	18.5 (0.7)	9
2,000 r.	12.4 (0.5)	39
4,000 r.	8.1 (1.1)	61
After 4 h assay		
—	18 (0.7)	—
500 r.	17.1 (0.9)	5
1,000 r.	17.1 (1)	5
2,000 r.	14.3 (0.6)	21
4,000 r.	10.3 (0.8)	43

Attacker cells were from CDF_1 mice challenged i.p. with 10^7 L5178Y/DIC viable cells and assayed after 10 d against ^{51}Cr-labelled L5178Y/DIC cells. Attacker cells were X irradiated (Securix Compact –CGR, 200 kV; 12 mA; filter Cu 0.5 mm, Al 0.5 mm, 23 cm distance, dose rate 98.2 r. min^{-1}) at 4° C. and the activity tested immediately or after 4 h incubation at 4° C.

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A lymphocyte-inhibiting factor isolated from normal human liver

WHILE we were looking for cell-mediated immunoreactions in patients with active chronic hepatitis (ACH), non-reproducible results with the leukocyte migration inhibition test[1], using

Table 1 Dose-dependent effect of inhibitor on PHA- and MLC-stimulated human lymphocytes and HeLa cells

Inhibitor (μg/0.2 ml)	Lymphocytes PHA (^{3}H-Thymidine Mikro) (Ly-control) (c.p.m.)	Inhibitor (μg/0.2 ml)	Lymphocytes MLC (^{3}H-Thymidine Mikro) (Ly-control) (c.p.m.)	Inhibitor (μg/0.2 ml)	HeLa ^{3}H-Thymidine (c.p.m.)	HeLa ^{3}H-Thymidine (c.p.m.)
0	8,442 (59)	0	7,319 (312)	0	77,000	42,000
—	—	8	5,116	6.2	78,000	33,000
12	2,923	12.5	1,590	12.5	80,200	34,000
16	102	—	—	—	—	—
25	26	25	288	25	61,300	27,000
50	39	—	n.d.	50	n.d.	22,000
100	28	—	n.d.	100	n.d.	12,000

different preparations of liver antigen from human liver, led us to attempt to prepare a better defined antigen from human liver—already proposed[2] for this test system. Besides a liver specific protein as previously described[11], our preparation yielded a soluble fraction, which inhibited the peripheral leukocytes of patients with active chronic hepatitis, but also inhibited the leukocyte migration of controls. The migration inhibition in these cases was therefore not due to a cell-mediated immune reaction against liver antigen, but was caused by a previously unknown inhibiting substance, extracted from human liver. Here we report some properties of this inhibitor.

The inhibitor was isolated from the soluble supernatant after homogenising and centrifugation of normal human liver at 100,000*g* for 90 min in Hanks solution, *p*H 7.2. The supernatant was transferred to a Sephadex G-100 column, equilibrated with 0.01 M Tris-HCl buffer, *p*H 8.0. The inhibiting factor was eluted in the second (II) and third (III) fraction. Rechromatography of these active fractions yielded a homogeneous peak, but no pure substance. An ion exchange chromatography procedure was therefore added, and we obtained a pure substance with inhibiting properties.

This inhibitor is a protein. The molecular weight, estimated by gel filtration, is about 65×10^3–8×10^3. The electrophoretic mobility is slow, similar to the β–γ-globulins.

The functional properties of this protein are characterised by a potent inhibition of lymphocyte transformation after stimulation by phytohaemagglutin (PHA), purified protein derivative (PPD) and by cellular antigens or transplantation antigens (mixed lymphocyte culture (MLC)) (Fig. 1). Besides, the metabolism of *in vivo* activated lymphocytes[3,4] and the migration of the macrophages are inhibited also. After PHA stimulation, most of all the *de novo* synthesis of RNA is inhibited.

The specificity of lymphocyte metabolism inhibition seems to be very high. Doses which cause a 100% inhibition of lymphocyte's metabolism lead only to a very slight reduction of the metabolic rate of HeLa cells (Table 1). On the other hand, no species specificity of the inhibitor could be found, since a preparation from rabbit liver has about 40% of the inhibiting potency on human lymphocytes compared with inhibitor prepared from human liver.

The inhibitor reported here is not identical with the immunosuppressive α globulins from human serum[6,7], because the inhibitor from human liver has an electrophoretic mobility of a β or γ globulin. We have isolated this inhibitor from the pooled serum of normal people also (by gel-filtration) but the inhibiting strength of this preparation was only 10% of that prepared from liver. We therefore suppose that the inhibitor is released from the liver to the serum.

Whether or not the prolonged survival time of skin allografts with simultaneous liver transplants on *Diemictylus viridescens*[7] was due to the immunosuppressive effect of the lymphocyte inhibitor from liver is unknown. The clear cut time dependency of liver implantation and immunosuppressive effect and the survival of the skin allograft does not exclude this possibility.

The inhibitor described here has some similarity with the inhibitor of protein synthesis in polyribosomes, reported by von der Decken[8]. The fact that the polyribosomes were prepared from rat liver, however, makes it unlikely that the inhibitor of protein synthesis in polyribosomes and our inhibitor are identical or similar. The inhibitor reported here seems to be lymphocyte specific.

The inhibitor is not a chalone[9], because chalones are defined as non species-specific inhibitors, present in and produced by the tissue, the proliferation of which they specifically inhibit[10].

The physiological significance of this inhibitor remains to be elucidated, especially the mode of action and the question whether or not this substance has something to do with regulation or repair mechanisms. Studies on the inhibitory effect on T or B lymphocytes are in progress.

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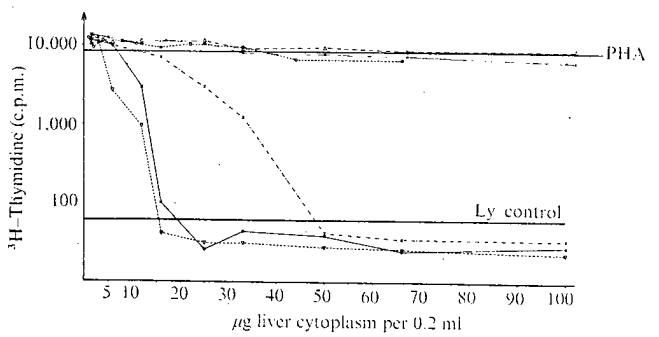

Fig. 1 Dose-dependent inhibition of PHA-stimulated lymphocytes by fractions (Sephadex G100) from soluble liver cytoplasm. ○ - - - ○, Fraction I; X · · — · · X, fraction II; ▽ - - - ▽, fraction III; X —— X, fraction IV; △ · — · △, fraction V; ●—●, liver cytoplasm.

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Two types of resistance to polyene antibiotics in *Candida albicans*

STUDIES reported during the past 15 years have led to the widely accepted conclusion that polyene antibiotics function

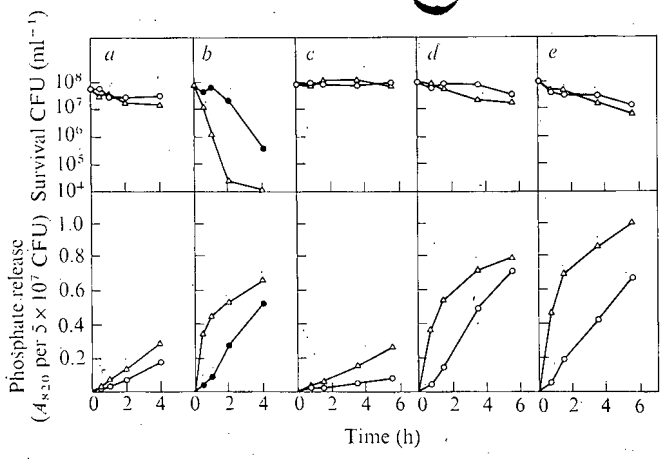

Fig. 1 The effect of polyene on the viability (CFU ml^{-1}) and on the release of phosphate ($A_{820\,nm}$ per 5 × 10^7 CFU) from mutants A1 (*a*), A2 (*b*), A3 (*c*), A4 (*d*) and A7 (*e*) at room temperature. Only exponentially growing cells were suspended in saline. After incubation with polyene for the periods shown, viability was determined by plate-counting and the release of phosphate was quantitated colorimetrically[21] on the supernatant fluid after centrifugation. The amount of release in the blank control samples remained negligible. ●, 10 μg amphotericin B ml^{-1}; ○, 40 μg amphotericin B ml^{-1}; △, 100 μg nystatin ml^{-1}.

by binding with the sterol molecules in susceptible cells to cause permeability change and eventual death of the cell[1-3]. In recent reports[4-6] based on liposomal model membrane studies, it has been suggested that susceptibility to polyene antibiotics is determined by organisation rather than by a single component of the membrane, namely sterol. Here, through study of polyene-resistant mutants, we further propose that, at least in some cases, permeability alteration may not be the specific critical event in the fungicidal action of polyene antibiotics.

Five mutagen-induced mutants were derived from a clinical isolate of *Candida albicans* (E139-A, wild type) as described by Hamilton-Miller[7]. Their levels of resistance vary with inoculum size and with the medium (Table 1). For example, mutant A2 grew in 100 μg ml^{-1} nystatin on solid medium but was killed in saline. Compared with E139-A, the mutants grew more slowly, responded differently in the Liebermann–Burchard colorimetric reaction (Table 1) and, in accordance with previous reports[8,9], contained proportionally more sterol (Table 2). In other laboratories, differences in the sterol pattern of polyene-resistant mutants have been observed[8-13], and the major sterols have been characterised completely in one set of mutants[14]. Polyene resistance was attributed to altered patterns of sterols. In several studies[10,11,13], decrease in ergosterol content has been singled out as the direct cause of polyene resistance. For comparison, the ultraviolet absorption spectra of the non-saponifiable fraction extracted from E139-A and our polyene-resistant mutants were examined[10]. In all cases, 5,7-diene sterols (for example, ergosterol and 24(28)-dehydroergosterol) were detected but not as major components. This was confirmed by analysis of the sterol composition with thin-layer chromatography (Table 3). A similar situation has been reported in a strain of *Saccharomyces cerevisiae*, in which ergosterol constituted only 10% of the total sterol[15].

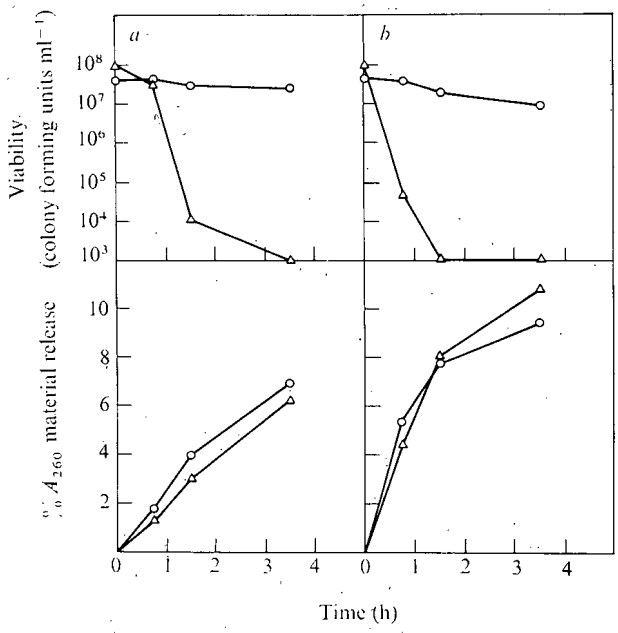

Fig. 2 The effect of polyene on the viability (CFU ml^{-1}) and on the release of ultraviolet-absorbing substances (260 nm) from the wild type E139-A (△) and from mutant strain A4 (○). Exponentially growing cells were used. In (*a*) amphotericin B was present at 20 μg ml^{-1}; in (*b*) nystatin was present at 100 μg ml^{-1}; in (*b*) nystatin was present at 100 μg ml^{-1}. Experimental conditions were identical to those described for Fig. 1. The maximal extractable ultraviolet-absorbing material from the cells was determined by treatment with 1.2 N perchloric acid[17] and taken as 100% release.

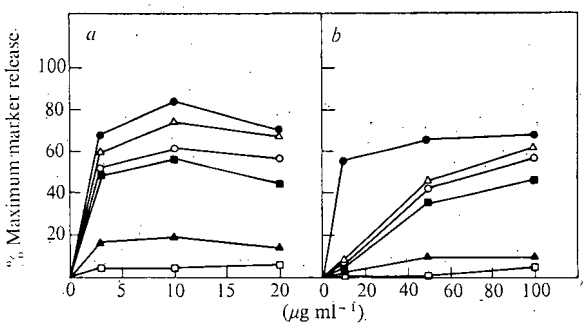

Fig. 3 Polyene (*a*, amphotericin B; *b*, nystatin) susceptibility of liposomes derived from total lipid extracts of 139 (●), A1 (□), A2 (■), A3 (▲), A4 (○) and A7 (△). Liposomes with the test agents, as well as the blank control, were incubated at room temperature for 30 min. The percentage maximum glucose released was calculated from the expression: (glucose released in the presence of polyene — blank control)/(total amount of glucose trapped — blank control) × 100, as described previously[4,6].

We have found that effects on viability and cell leakage depend on temperature and phase of growth. At room temperature (23°–24° C), nystatin and amphotericin B caused extensive killing and leakage of phosphate and ultraviolet-absorbing material from E139-A. At 2° C, however, no cells were killed and we observed no leakage of phosphate and ultraviolet-absorbing material from them. Similarly, cells in stationary

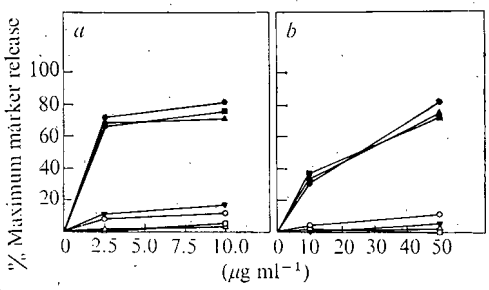

Fig. 4 Polyene (*a*, amphotericin B; *b*, nystatin) susceptibility of mixed lipid liposomes. Phospholipid and neutral lipid derived from one strain were mixed, respectively, with the neutral lipid and phospholipid derived either from the same strain (control liposome) or from a different strain (mixed lipid liposome). Experimental conditions were identical to those described for Fig. 3. Neutral lipids of: ●, E139-A; ■, E139-A; ▲, E139-A; ▼, A1; ○, A1; □, A3; △, A3. Phospholipids of: ●, A1; ■, A3; ▲, E139-A control; ▼, E139-A; ○, A1 control; □, E139-A; △, A3 control.

Table 1 Characterisation of polyene-resistant mutants of *C. albicans*

		Polyene added ($\mu g\ ml^{-1}$)	Strain	E139-A	A1	A2	A3	A4	A7
	Blank control	0.0		‡	†	‡	†	‡	†
	Amphotericin B	40.0		—	—	—	—	—	—
		20.0		—	*	—	*	—	—
		10.0		—	†	—	†	*	—
		2.5		—	‡	—	†	‡	—
Growth in		0.5		—	†	‡	†	‡	†
liquid medium*	Nystatin	100.0		—	†	—	†	—	—
		50.0		—	†	†	†	‡	†
		25.0		—	†	‡	†	‡	†
		10.0		*	†	‡	†	‡	†
		2.5		‡	†	‡	†	‡	†
	Nystatin	100.0		—	*	*	*	*	*
Growth on	Nystatin*	100.0							
solid medium†	Amphotericin B	20.0		—	*	—	*	*	—
Colour reaction‡				Greenish blue	Bright yellow	Purplish blue	Bright yellow	Greyish blue	Greyish blue
Doubling time (min)§				65	85	70	97	90	135

* Log phase cells were adjusted to approximately 5×10^4 colony forming units per ml in fresh growth medium; 2 ml of this dilution was added to tubes containing the given polyenes. Dimethyl sulphoxide was present to 1% in all samples including the blank control. After overnight incubation at 37° C, the turbidity was recorded.

† A single colony of the given strain was streaked out on plate containing the given polyenes, and the growth was checked after 24 h of incubation at 37° C. The nystatin (4530 U ml^{-1}) and the amphotericin B (876 $\mu g\ ml^{-1}$) were gifts from the Squibb Institute of Medical Research.

‡ Liebermann–Burchard reaction used. Total lipid extract of whole cells were used for assay. Preparations from both log and stationary phase cells gave identical results.

§ Growth was monitored turbidimetrically with a Klett–Summerson photoelectric colorimeter (green filter).

phase were affected far less than those in the logarithmic phase. (Unless otherwise stated, only exponentially growing cells were used subsequently.) These temperature and growth phase effects might be explained in three ways: (1) changes of chemical composition in the membrane; (2) changes of effective pore size cell wall (a molecular sieving effect[16]); (3) a temperature-dependent metabolic process, operative only in growing cells, may be involved in the fungicidal action. No decisive evidence favours any of these suggestions, and temperature dependence and growth phase dependence could be unrelated. But, we have observed no appreciable difference in sterol patterns nor in the polyene-sensitivity of liposomes prepared from growing and stationary cells. Furthermore, protamine (molecular weight approximately 18,500) can release ultraviolet-absorbing material from a strain of *C. utilis* at both 30° C and 0° C (ref. 17).

Figure 1 shows the relationship of viability and leakage in mutants. The release of cellular constituents did not result in or parallel extensive killing in mutants A4 (Fig. 1*d*) and A7 (Fig. 1*e*). The latter phenomenon is illustrated more clearly in Fig. 2. In the presence of amphotericin B and nystatin, the viability of A4 was maintained whereas E139-A was soon killed; the release of ultraviolet-absorbing material from both strains remained comparable. If the alteration in permeability is a direct measure of membrane–polyene interaction, the data in Figs 1 and 2 suggest that there may be two mechanisms of polyene-resistance in yeast. With type 1 mutants, A1 and A3, resistance arises from a reduced interaction between the cell membrane and the antibiotics as indicated by the low level of leakage. With type 2 mutants, A2, A4 and A7, the antibiotics interact with the membranes, as evidenced by the altered permeability, but some critical step in the fungicidal effect fails to occur.

The categorisation of mutants based on the loss of cell constituents can be reproduced in liposomal membrane systems derived from the total lipid extract of each strain (Fig. 3). Liposomes prepared from mutants A2, A4 and A7 leaked glucose in a manner comparable with the wild type. The apparent decrease in glucose release at high concentrations of amphotericin B was due to the interference of the antibiotic molecule at the absorption wavelength used in our assay. Mixed liposomes were also prepared after fractionating total lipid extracts into phospholipid and neutral lipid components by silicic acid column chromatography[18]. Figure 4 shows that polyene-induced permeability of E139-A was associated

Table 2 Incorporation of sodium acetate-1-^{14}C into lipid fraction of wild type and mutant strains

	% Distribution of phospholipids			Ratio of phospholipid/ neutral lipid*
	PC	PE	PS+PI	
E139-A	73.8	20.9	5.3	2.41
A1	60.6	28.0	11.4	1.74
A2	69.5	22.1	8.3	1.62
A3	68.7	19.9	11.3	1.63
A4	70.7	22.4	7.0	1.92
A7	76.5	13.1	10.4	1.46

Total lipid extract was subject to thin-layer chromatography on silica gel G plates with system $CHCl_3/CH_3COOH/H_2O$/AcOH (65:43:3:1, v/v). Percentage distribution of phospholipids was calculated from the expression: [(c.p.m. of given phospholipid) (total c.p.m. in phospholipid fraction)] × 100. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol.

* Represent ratios of c.p.m. in phospholipid fraction to c.p.m. in neutral lipid fraction. Preliminary experiments indicated that about 90% of the counts in neutral lipid fraction was associated with free sterols.

Table 3 Mobility and percentage distribution of major sterol classes of wild type and mutant strains on thin-layer chromatography

	R_F values					
	<0.14	0.32	0.48	0.55	0.67	0.72
E139-A	30.6	18.1		11.7	29.7	10.0
A1	38.8	8.8		18.2	22.4	11.8
A2	24.2	5.7	12.4	23.6	25.9	8.3
A3	45.2	7.4		15.5	21.2	10.8
A4	36.9	10.8		17.2	25.5	9.7
A7	24.4	13.3		22.3	27.4	12.7

Non-saponifiable sterol extracts were subject to thin-layer chromatography on $AgNO_3$-impregnated silica gel G plates. The plates were developed half-way with benzene–ethyl acetate (2:1, v/v), dried under N_2 and redeveloped in the same direction the full distance with benzene–ethyl acetate (5:1, v/v). Figures given were calculated from the expression: [(c.p.m. of a given spot)/(total c.p.m. in sterol fraction)] × 100.

* As references, the R_F values of ergosterol and cholesterol are 0.32 and 0.55, respectively. Sterols with polarities (as measured by their R_F values on silica gel) greater than ergosterol were not well resolved in this system.

with the neutral lipid fraction (shown to be predominantly free sterol in preliminary experiments). Similarly the insensitivity of A1 and A3 to polyene-induced permeability changes was conferred on mixed liposomes by the neutral lipid fraction of these organisms. Our results clearly show that, in type 1 mutants, resistance arises from changed sterol patterns. Structural characterisation of major sterols in all strains is needed before this type of resistance can be discussed more specifically.

The existence of type 2 mutants indicates that leakage does not always result in killing. The discovery of nystatin-dependent mutants[19] suggests that this antibiotic could play a role in cell physiological function. The lethal action may involve polyene-induced malfunction of an essential physiological process or processes. When such a process is not operative, for example, at 2° C or when cells enter stationary phase or when a specific mutation occurs, its susceptibility to the fungicidal effect of polyenes may diminish. It has been reported[20] that iodoacetate and dinitrophenol reduce the fungicidal action of the polyenes on yeast.

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Crystallisation of a modified fibrinogen

Most fibrous proteins do not crystallise. An artful way to promote protein crystallisation is to modify the structure by proteolytic attack. For example, papain cleavage of γ-globulin yields the homogeneous Fc fragment which readily crystallises[1]. Following a similar approach, we have crystallised fibrinogen.

Native fibrinogen precipitated at low ionic strength forms fibrous aggregates which are aperiodic in the electron microscope. After these precipitates had been stored in the cold for several months, we observed unusual rod-shaped structures which showed microcrystalline order in the electron microscope[2]. We had previously noticed that such preparations were often contaminated with bacteria, and it seemed likely that a bacterial protease had modified the native molecule so that it formed ordered aggregates. We cultured strains of *Pseudomonas aeruginosa* from such preparations and prepared a crude protease extract. This protease cleaved fibrinogen to a form that yielded highly ordered microcrystalline arrays[3]. We summarise here our studies on the effect of the protease digestion on fibrinogen and show that more extensive controlled cleavage leads to a modified molecule which forms crystals suitable for X-ray diffraction analysis.

The protease used in these studies was isolated from the growth medium of a strain of *Pseudomonas aeruginosa* grown on trypticase soy. The crude enzyme was salted out with ammonium sulphate. The protease was incubated at 31° C with native fibrinogen (5 mg ml^{-1} in 0.3M NaCl buffered to about *p*H 7) for various times. The reaction was terminated by addition of 10^{-3}—10^{-4}M N-α-Tosyl-L-lysine chloromethyl ketone (TLCK), and the fibrinogen was dialysed to low ionic strength (5×10^{-3}M KSCN, 0.01M 2-(N-morpholino) ethane sulphonic acid (MES), *p*H 6.2). Samples were stored in the presence of the inhibitor and chloroform.

The progressive modification of the molecule was followed by measuring the percentage of mass lost as small trichloroacetic acid (TCA)-soluble peptides, and by observing the type and amount of ordered aggregates formed at low ionic strength. Four stages of digestion were distinguished. In the very early phase, corresponding to less than about 2% mass loss, most of the material precipitated out of solution at low ionic strength and consisted chiefly of aperiodic fibres, similar to native fibrinogen. With further digestion (corresponding to a loss of 4–6% of the mass as TCA-soluble material) the modified fibrinogen was more soluble, but within about 3 d as much as 85% of the material precipitated as microcrystals with a 450 Å axial repeat[3]. In the narrow digestion range between about 6 and 8% mass loss, precipitation was even slower, and both microcrystals and large birefringent crystals appeared; the proportion of crystals could be very high. On further digestion (about 10% mass loss) some amorphous precipitate formed, but most protein remained in solution.

The extent of protease digestion could be correlated with the appearance of the sodium dodecyl sulphate (SDS) gel electrophoresis patterns run in the presence of reducing agents[4]. The native molecule has a molecular weight of about 340,000 and consists of three pairs of chains designated Aα (~70,000 daltons), Bβ (~60,000 daltons) and γ (50,000 daltons)[5,6] (Fig. 1*c*). The Aα chain was attacked first. Microcrystalline samples, digested to about 4% mass loss, gave gel patterns with most of the Aα band and part of the Bβ band missing. The gel patterns of crystals showed a single heavily staining broad band with the same mobility as the γ chain of the native molecule (Fig. 1*c*). The clotability by thrombin of the molecules from the crystals was about 50% to 80% of that of native fibrinogen.

The crystals we obtained were thin birefringent plates with the longest dimension up to 1 mm (Fig. 1*a*). They were very fragile and characteristically cleaved in a direction perpendicular to the long axis to give rod-shaped fragments.

Preliminary X-ray diffraction patterns of single crystals taken at room temperature show reflections out to about 3 Å (Fig. 1*b*). The space group is $P2_1$. The unit cell dimensions are a=134 Å, b=97.3Å, c=174Å. β=92°20′. The density of the crystals is in the range 1.135–1.140 g cm^{-3} as determined by flotation of individual crystals in xylene–bromobenzene mixtures. Assuming that the partial specific

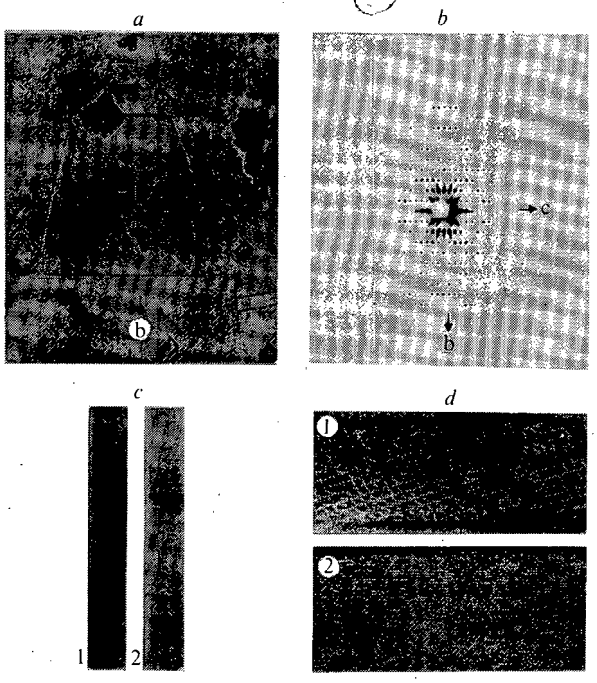

Fig. 1 *a*, Crystal of proteolytically modified fibrinogen. The b-axis is parallel to the long (vertical) axis of the crystal. Note small fragments cleaved perpendicular to this direction. (Approximately ×80.) *b*, A 4½° Precession photograph of of the Okl plane of the reciprocal lattice. The systematic absence of the OkO reflections with k odd indicates a two-fold screw along the b-axis (vertical direction). *c*, SDS polyacrylamide gel electrophoresis patterns of (1) native fibrinogen; (2) modified fibrinogen from redissolved crystals. Both reduced with mercaptoethanol. *d*, Electron micrograph of crystal fragments showing (1) a net-like region: (2) fine diagonal, lateral and longitudinal striations spaced at about 45 Å, 60 Å and 35 Å, respectively. The sides of the mesh are about 105 Å by 115 Å; the acute angle is about 65°. Samples negatively contrasted with 1% uranyl acetate. (×190,000.)

volume of the protein is 0.72[7], the water content of the crystals is about 65%. The molecular weight of the protein in the asymmetric unit was therefore calculated to be 330,000, with an uncertainty of about 6%.

Crystals were washed, fragmented and negatively contrasted with 1% uranyl acetate for electron microscopy. Commonly observed images showed fine diagonal and longitudinal striations spaced at about 45 Å and 35 Å. More rarely, a net-like form was seen (Fig. 1*d*). The beaded strands of the mesh were about 30 Å in diameter, with the edges about 115 Å by 105 Å in length, defining an acute angle of about 65°. The negatively stained crystal fragments showed no direct correspondence with images of the microcrystalline forms which have a 450 Å axial repeat[3].

The unit cell dimensions and density of the crystals indicate a molecular weight for the modified protein of about 330,000 comparable with the native molecule. The estimate of mass lost from modified fibrinogen which crystallises is about 6–8% by measurement of the TCA-soluble peptides released during digestion. SDS gels of the crystals run in reducing conditions, where polypeptide chains separate by molecular weight, show material only in the γ chain region. Since no other large chain fragments are seen, the cleavage may split off relatively small fragments from the Aα and Bβ chains, reducing them to the size of the γ chain. The γ chain has a molecular weight of about 50,000; thus the weight inferred for the modified molecule from these gels is again about 300,000. Migration of the whole modified molecule on SDS gels without reducing agents present also gives a rough estimate of molecular weight close to 300,000. The chemical and crystallographic estimates of the mass of the modified molecule thus agree, within experimental uncertainties. This preliminary evidence on the modified molecule forming the crystal indicates that in spite of the cleavage it may be largely intact.

Redissolved crystals show a clotability about 50–80% of that of native fibrinogen. The fibres formed appear very similar to those of native fibrin in the electron microscope. This indicates that the modified molecule has retained the critical sites which interact to form the fibrin clot.

Some inferences about molecular shape and packing can be made at this stage, although the crystallographic data do not provide strong constraints. The modified fibrinogen crystallises in the monoclinic space group $P2_1$, and the unit cell contains two molecules of weight about 300,000. The molecule is a dimer of elongated shape, but a twofold axis is not a symmetry element of the crystal. From the symmetry of the crystal, these rod-shaped molecules must be arranged with two different orientations. Since the crystals readily cleave along the plane perpendicular to the b axis, the forces are weak between arrays of molecules related by the twofold screw. Only about 35% of the unit cell volume is occupied by protein, so that the molecules must form a fairly open meshwork of cross-connected rods.

The body diagonal of the unit cell in the crystal is about half the 450 Å length inferred for the fibrinogen molecule from studies of the microcrystals[3]. The dimensions of the unit cell in the crystal lattice need not, however, be related simply to the molecular dimensions. As in the assembly of other fibrous proteins[8–11] the rod-shaped molecules may pack with extensive side-to-side overlaps and end-to-end gaps, so that the observed periods may be only a fraction of the molecular lengths. The microcrystals of the modified fibrinogen molecule display a 450 Å axial period, and a specific staggering of arrays with this period can produce the 225 Å axial period and density distribution seen in the fibrin clot[3]. Although these large periodicities are not evident in the crystal lattice, it is likely that the modified fibrinogen in these crystals will show interactions similar to those in the microcrystals and in fibrin.

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(1497)

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At the Department of Mines, Addis Ababa, to be responsible for organising and establishing a comprehensive record system, and the training of local staff. Applicants must have a degree or equivalent qualification in Geology or Mining Engineering. Subsequent experience of the mineral industry, including the use of data processing methods essential. Appointment for 1 year.

Salary in range £4,400 to £5,200 pa plus a tax free overseas allowance in range £895£ to 1,995 pa.

Other benefits include free family passages, paid leave, children's educational allowances, and free accommodation and medical attention. Superannuation rights may be safeguarded and all emoluments are paid by the British Government. Applicants should normally be citizens of, and permanently resident in, the United Kingdom.

For full details and an application form please apply giving age and details of qualifications and experience to:

Appointments Officer
Ministry of
Overseas Development
Room 301
Eland House
Stag Place
London SW1E 5DH (1466)

FOOD MICROBIOLOGIST

The Union International Co. Ltd. has a vacancy for a Graduate Food Microbiologist at the St. Albans Research Centre.

The work is varied and interesting, and will include investigations into vacuum packed and cured meats.

Preference will be given to candidates who have some practical experience in meat microbiology.

A certain amount of travelling will be involved, visiting plants in the U.K.

Applications giving full personal and career details should be sent to the Staff Manager (AD 5849), 14 West Smithfield, London EC1A 9JN.

(1493)

BIOCHEMIST

A Research Assistant is required to investigate the microbial metabolism of detergent products, in particular, the metabolic pathways used during their biodegradation in the environment.

This post would be suitable for a recent graduate Biochemist with an interest in mircobiology and some concern for environmental issues.

The Laboratory is situated on the Wirral Peninsula, a few miles from the sea and convenient for Chester, Liverpool and North Wales.

Interested persons should write for an Application Form quoting reference No. PS467AM to Personnel Department, Unilever Research, Port Sunlight Laboratory, Port Sunlight, Merseyside L62 4XN.

(1532)

Micro-Biologist

A Micro-Biologist, graduate level, to join a small team servicing international product research on proprietary medicines, food, toiletry and cosmetic products. Vitamin bio-assay experience an advantage. Good opportunities for a young graduate with management capacity.

Benefits include free pension and life assurance schemes.

Please apply in writing quoting qualifications and brief career details to:

Personnel Manager
Beecham Products Research Dept.,
Randalls Road, Leatherhead, Surrey.

Beecham Products

(1483)

UNIVERSITY OF OTAGO
DUNEDIN, NEW ZEALAND
CHAIR OF HUMAN NUTRITION

The University Council invites applications from medical or science graduates for the foundation Chair of Human Nutrition.

Applications will be welcomed from candidates with interests in any field of Human Nutrition.

Professorial salaries are fixed at various points within the present range of NZ$15,111 per annum to NZ$19,233 per annum, and are regularly reviewed.

Further particulars are available from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close in New Zealand and London on **October 31, 1974.** (1488)

ACADEMIC POSITION

One immediate opening for a Ph.D. chemist, preferably in the area of analytical chemistry with research interest in biomedical applications. Duties include graduate and undergraduate teaching and establishing a vigorous research programme. Rank open. This position has been created through a grant from the Minority Schools Biomedical Support Programme of the National Institutes of Health. Successful applicants will be required to interact strongly with minority students in this programme. Send résumé to: Dr Ralph G. Wilkins, Head, Department of Chemistry, Box 3C, New Mexico State University, Las Cruces, New Mexico 88003. New Mexico State University is an equal opportunity and affirmative action employer.

(1489)

UNIVERSITY OF LEEDS
DEPARTMENT OF FOOD AND LEATHER SCIENCE

Applications are invited for the post of **RESEARCH ASSISTANT** to take part in a basic research programme on the relation between the properties, structure and method of manufacture of leather.

An interest in materials science, especially in mechanical behaviour, with an appropriate degree qualification is required but no previous experience with leather is necessary. The post is for one year in the first instance renewable for a further two years. Salary within the range £1,638 to £2,007. Applications giving the names of three referees should be sent to Professor A. G. Ward, Procter Department of Food and Leather Science, University of Leeds, LS2 9JT by October 25. (1479)

UNIVERSITY OF SYDNEY
PROFESSOR/ASSOCIATE PROFESSOR IN BEHAVIOURAL AND SOCIAL SCIENCES
(In Relation to Medicine)

Applications are invited for the position of Professor/Associate Professor in Behavioural and Social Sciences (in relation to Medicine). The appointee will be responsible for the direction of a new course, introduced in 1974, designed to give medical undergraduates a substantial orientation to the behavioural and social sciences as they relate to health, disease and medical practice.

The salary for a Professor is $A19,614 per annum and for an Associate Professor is $A16,389 per annum.

A statement of Conditions of Appointment and Information for Candidates may be obtained either from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF or from the Registrar, University of Sydney, N.S.W. 2006, with whom applications close on **November 22, 1974.** (1487)

TRANSLATORS

to prepare English summaries of German and French texts required full time by Woolcott & Company, 93 Chancery Lane, London WC2. Salary £2,500 p.a. plus according to age and experience (1480)

PLANT BREEDING INSTITUTE
CAMBRIDGE

CONTROLLED ENVIRONMENT ENGINEER

An engineer is required to take charge of the running and maintenance of controlled environment installations at the Institute. He will also be required to operate the installations to maintain the environmental regimes needed for crop research. He will be responsible to the Head of the Physiology Department.

Candidates should have experience of environmental engineering, air conditioning, and electrical circuits. They must be able to keep detailed records of maintenance schedules and environmental data.

Candidates should have a degree in a scientific or engineering subject; degree-standard membership of a professional institution; a Higher National Certificate or Higher National Diploma; or qualifications equivalent to at least H.N.C.

The post will be filled at the grade of Scientific Officer (£1,592 to £2,675 per annum) or Higher Scientific Officer (£2,461 to £3,371 per annum). Grading and starting salary will be determined in relation to qualifications and experience. Non-contributory pension scheme. Candidate for the higher grade should have had at least five years appropriate experience since qualifying.

Further particulars may be obtained from the Assistant Secretary (Establishment), Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ. Applications including curriculum vitae and the names and addresses of three referees should reach him not later than October 31, 1974. (1506)

AUSTRALIAN NATIONAL UNIVERSITY

FACULTY OF SCIENCE

CHAIR OF FORESTRY

The Chair of Forestry will become vacant in late October 1974 when Professor J. D. Ovington, the foundation Professor, resigns in order to take up a position with the Australian Government.

The appointee would for an initial period of some years be Head of the Department. The University would regard as essential the appointment of a person who would preserve the current emphasis on the multi-disciplinary nature of the Department's interests and who would maintain rapport with the forest services and with industry.

The salary for professor is $A19,614 p.a.

OTHER CONDITIONS—Tenure of Professor is to retiring age (65 years).

Reasonable travel expenses are paid and assistance with housing is provided for an appointee from outside Canberra. Superannuation is on the F.S.S.U. pattern with supplementary benefits.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should write to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF for further particuars before appying.

Closing Date: **November 18, 1974.** (1486)

PALEOSERVICES LTD.

requires a

PALYNOLOGIST

with Tertiary and Mesozoic interest (two to three years postgraduate experience desirable).

Apply to: Paleoservices Ltd., Paramount Industrial Estate, Sandown Road, Watford, Herts. WD2 4XA. Tel: Watford 25678. (1503)

CHELSEA COLLEGE
University of London

BIOPHYSICS LABORATORY
GRADE 3 TECHNICIAN

required to assist in research and teaching activities within the Biophysics Section of the Basic Medical Sciences Group. Main duties will be associated with research into photobiological processes and artificial membrane systems. Experience of biophysical and/or biochemical techniques would be an advantage. Salary Scale: £1,878 to £2,148 (including £228 London Allowance) plus Threshold Payments. Enquiries and application forms from Dr W. P. Williams, Biophysics Laboratory N, Chelsea College, Manresa Road, London. SW3 6LX (01-352 6421). (1484)

SCOPE/ICSU
MONITORING CENTRE
CHELSEA COLLEGE
UNIVERSITY OF LONDON

SCIENTIFIC PROGRAMME OFFICER

Applications are invited for a physical or biological scientist with substantial post-graduate experience (5–8 years) who is prepared to accept administrative, executive and liaison responsibilities within an international research group. The group is concerned with Environmental Monitoring and Assessment and forms part of the activities of the Scientific Committee on Problems of the Environment (International Council of Scientific Unions).

The appointment is for 1 year renewable for up to 2 years and will be within the University of London at the upper end of the Lecturer Scale or within the Senior Lecturer/Reader level (£2,580 to £5,976 plus £213 London allowance), depending upon age, qualifications and experience. Duties will include liaison with international scientific groups, the organisation of scientific workshops and symposia, co-ordination of the research group activities and provision of administrative assistance to the director of the group.

Fluency in French would be an advantage.

Further particulars of the above post and conditions of appointment can be obtained on request from the Appointments Officer, Chelsea College, Manresa Road, London SW3 6LX, to whom applications including names of two referees should be sent by November 11. (1481)

UNIVERSITY OF MALAYA

FACULTY OF ENGINEERING

Applications are invited for the posts of

LECTURER/ASSISTANT LECTURER

in the fields listed below in the Faculty of Engineering. Candidates should have at least a good Honours degree in Engineering, Mathematics or Physics, teaching at degree level would be an advantage.

Subject to suitable academic qualifications and experience, preference will be given to candidates who are proficient in Bahasa Malaysia (Malay) but this requirement is not applicable to overseas staff. If selected, overseas candidates may be offered a short-term contract subject to the possibility of renewal by mutual agreement.

Salary scales (approx. stg. equivs.): Lecturer £1,524 to £2,441; Assistant Lecturer £1,330 to £1,460 p.a. In addition the following allowances are payable: Variable Allowance £243 min. £649 max. p.a. calculated at 35% of basic salary. Supplementary Housing Allowance £503 p.a. and medical benefits.

Further particulars and application forms are obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

The closing date for the receipt of applications is **November 14, 1974.** (1512)

UNIVERSITY OF THE WITWATERSRAND
Johannesburg, South Africa

SENIOR LECTURER/LECTURER IN GENETICS

Applications are invited for appointment to the above post in the newly established Department of Genetics. The ability to teach Population Genetics and a research interest in Molecular Genetics will be a recommendation.

The salary attached to the post presently falls within the following ranges:

Senior Lecturer	R7,245 to R9,315
Lecturer	R5,520 to R7,935

Improved salary scales are to be introduced shortly. Benefits include an annual bonus, pension and medical aid facilities, and a housing subsidy (where applicable).

Intending applicants should obtain the information sheet relating to this post from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa, with whom applications should be lodged not later than November 20, 1974. U.K. applicants may obtain the information sheet from the London Representative, University of the Witwatersrand, 278 High Holborn, London, W.C.1 to whom a copy of the application should be sent. (1514)

THE UNIVERSITY OF NEW SOUTH WALES
WOLLONGONG UNIVERSITY COLLEGE
To become the UNIVERSITY OF WOLLONGONG 1st January 1975
PROJECT SCIENTIST

Applications are invited from Ph.Ds. with a background in Quantum Chemistry for the position of Project Scientist in the Department of Chemistry. The successful applicant will be expected to work for Dr P. G. Burton on a project involving studies of the influence of the environment on the chemical reactivity in molecules in condensed media. New theoretical methods of electronic structure computation will be used and a detailed working experience with large Flortran programmes will be an advantage. Of particular interest are transition metal systems and the study may impinge on areas of inorganic photochemistry and of functions of metalloproteins.

Appointment in the first instance will be for up to 14 months beginning immediately with initial salary Aus.$9,002.

Further details of the project are obtainable from Dr Burton and applications should be made immediately to the College Bursar, Wollongong University College, P.O. Box 1144, Wollongong, N.S.W. 2500, Australia by November 22, 1974.

Applicants should request two referees to write immediately to the College Bursar. (1502)

NATIONAL INSTITUTE OF TECHNOLOGY, MALAYSIA
Kuala Lumpur

Appointments of

LECTURERS/SENIOR LECTURERS

in the following disciplines:

Engineering (a) Mechanical
(b) Electrical
(c) Civil

Surveying (a) Photogrammetry
(b) Geodesy
(c) Cartography
(d) Estate Management
(e) Hydrographical

Applications are invited for the above appointments tenable as from May 1, 1975. Applicants for the above appointments should possess high academic qualifications and have considerable teaching experience in the academic disciplines concerned and for Engineering, in the fields as follows:

Mechanical Engineering
Production Engineering: machine tools; metrology Thermodynamics: Heat and Mass transfer Control Engineering: Mechanical bias Automotive Engineering: Structures and dynamics Mechanics of Materials and metallurgy; Mechanical Design

Electrical Engineering
High Voltage; control; electrical machines; computer; communications: transmission and broadcasting; Industrial Electronics

Civil Engineering
Structural Design and Analysis
Soil and Foundation Engineering
Hydraulics and Hydrology
Environmental Engineering

SALARY: Senior Lecturer—(M)$1,405 by 55 to 1,735/RP/1,800 (Basic). Lecturer—(M)$940 by 45 to 1,210/1,255 by 50 to 1,505 (Basic).
Points of entry will depend on qualifications and experience.

OTHER PRIVILEGES will include variable allowance calculated at 35% of basic salary subject to certain maxima, superannuation benefits on the basis of contribution of 5% of basic salary by appointee and 15% of basic salary by the Institute, leave, medical benefits, housing allowance at (M)$310 per mensem, and, in the case of appointees on overseas terms, air passages for the appointee, his wife and dependent children on appointment and on completion of contract. Candidates, if appointed, will be on contract for 36 months in the first instance.

All applications must be made in writing giving full personal details and should also include the names of two referees.

Further information on the terms and conditions of service may be sought from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or obtained directly from:

The Registrar,
National Institute of Technology,
Jalan Gurney,
Kuala Lumpur (15-02),
Malaysia. (1494)

Recently Qualified Veterinarian

...to join a team of research scientists involved in the screening and identification of new compounds active in the field of growth promotion, feed utilisation and parasitology. He will be responsible for a section of research work in the growth promotion field and will also have general responsibility for the health of both research and farm animals.

The Thurgarton Research Station is located in rural surroundings between Nottingham and Southwell and has excellent field and laboratory facilities alongside the Boots farms which provide opportunity for extensive field testing. Later stages of research also involve trials throughout the U.K. and in overseas locations.

Terms and conditions of employment are attractive and include profit sharing and help with re-location. There is ample scope for career development in both the scientific and commercial sectors.

Further information on the position involved can be provided on request. Interested applicants should apply with details of their career to date to:

J. F. Pattison, Employment Manager,

Boots

The Boots Company Ltd.,
Station Street, Nottingham NG2 3AA.
Tel: Nottingham 56255 Ext. 243.

(1510)

THE MIDLAND CENTRE FOR NEUROSURGERY AND NEUROLOGY
Holly Lane, Smethwick,
Warley, Worcs. B67 7JX

DEPARTMENT OF NEUROPATHOLOGY

TECHNICIAN IN HISTOLOGY

Applications are invited for the post of Technician in Histology. The vacancy offers excellent opportunities for further study and post H.N.C./A.I.M.L.T. experience in all aspects of histology and is particularly suitable for an Associate studying for the Special examination in histological technique. The Department is well equipped and a wide range of techniques are utilised including histochemistry and electron microscopy. Candidates should possess A.I.M.L.T. or H.N.C. but consideration will be given to holders of O.N.C. Laboratory may be viewed by appointment.

Applications with the names and addresses of 2 referees to the Hospital Secretary at the above address. (1529)

Young Graduates for Research

In the Royal Dutch/Shell Exploration and Production Laboratory at Rijswijk, near The Hague, research is carried out in connection with the exploration for and recovery of oil and natural gas. Research work is directed towards improving techniques and methods applied in exploration, drilling and production, which calls for active co-operation with operating companies in solving their field problems. Work is done in project teams which are often made up of people of different disciplines.

Due to increased activity we need additional scientific staff, male or female, with preferably a PhD, to fill vacancies in the following fields:

General Research and Mathematics

Fundamental research in the rheology of rocks and granular materials, interfacial chemistry of liquid/liquid and liquid/solid contacts. Development of mathematical models for field development, particularly in deep water. Mathematical and computational service projects in support of research in other departments.

We require a classical experimental physicist, preferably in the field of solid and fluid mechanics, a colloid chemist and an applied mathematician with a background in operations research.

Geophysics

Developing and improving techniques for seismic exploration. Signal processing with the aid of computers, and interpretation of results. Study of the relation between seismic reflection and the actual geology of the area.

We require a physicist with a keen interest in digital processing and filtering techniques. Acoustics, information theory, signal theory and electronics are relevant fields of interest.

Petrophysics

Improvement and innovation of physical formation evaluation techniques and their interpretation in terms of formation characteristics, such as lithology, grain and pore-size distribution, pore fillers, cementation etc. Establishment of relations between production qualities of the reservoir and formation characteristics.

A good background in mathematical physics, both classical and modern, together with some experimental bias is required.

Production Geology

Geological studies of oil and gas reservoirs e.g. sedimentology, diagenesis, depositional tectonics. Analysis of petrophysical, mineralogical and sedimentological data to translate these into measurable parameters that will lead to better evaluation of reservoir character and distribution.

We require a general geologist and a geologist with experience in the physical chemistry of organic shales.

Production Chemistry

Studying problems of a chemical and physico-chemical nature encountered in drilling and completion of oil, water and gas wells. In particular, investigations of chemical methods of controlling sand influx into oil and gas wells, laboratory investigations and interpretation of results. Designing and organising field tests in co-operation with drilling and production experts. Study of technological problems in commissioning wells and in oil and gas production.

Chemists or chemical engineers are required who have a sound knowledge of physical and inorganic chemistry and are capable of making an engineering approach to field problem solving. A good understanding of the rheology of non-Newtonian fluids would be an advantage.

Oil and Gas Reservoir Engineering

Improvement of recovery from oil and gas reservoirs, involving fluid flow in porous media, interfacial phenomena in gas/liquid/solid systems, heat and mass transfer, thermodynamics of gas/liquid systems. Studies are experimental and/or are made with the aid of numerical simulators.

Both physicists with an interest in mathematical models and mathematicians to work on the development and extension of such models are required.

We offer training opportunities within and outside your professional field, flexible working hours to meet your personal preference and the possibility of overseas assignments.

Travelling and other reasonable expenses will be paid for interviews in London and The Hague. Financial assistance will be given for the removal of household and personal effects to The Netherlands.

Please write, with full details, to Shell International Petroleum Company Limited, Recruitment Division (A), PNEL/31, Shell Centre, London SE1 7NA.

Shell

(1525)

OXFORD POLYTECHNIC

Applications are invited for the post of

Lecturer II in Mammalian Physiology

The successful applicant will be expected to contribute to the teaching of Mammalian Physiology in the Modular (CNAA) degree course and HNC courses. An ability to teach the Cell Physiology and/or Pharmaecology could be an advantage.

Further details and application forms from
Dr R. E. Yorke,
Department of Science,
Oxford Polytechnic,
Oxford. OX3 OBP. (1467)

ANGLIAN WATER AUTHORITY

WELLAND AND NENE RIVER DIVISION

BIOLOGIST

Salary: Grade AP IV/V (£2,394 to £3,018)

There is a vacancy for a Biologist within the existing establishment of the Welland and Nene River Division of the Anglian Water Authority. This appointment will be based initially upon the existing laboratory at Oundle, and a major responsibility of the post will be water quality control for the Empingham Pumped Storage Scheme—involving intensive hydrobiological studies of the reservoir and supply rivers.

The successful applicant will be a university graduate and/or a member of the Institute of Biology and previous experience in this field is desirable. An essential car user allowance will be paid.

Applications giving details of age, qualifications, training and experience, together with names and addresses of two referees should reach the Divisional Manager, Welland and Nene River Division, Anglian Water Authority, North Street, Oundle, Peterborough, by November 11, 1974.

(1537)

CHIEF TECHNICIAN

Applications are invited for the post of Chief Technician to be in charge of the serological reagents and standards section. An extensive knowledge of all aspects of blood group serology is essential and experience of automation would be an advantage. Salary on Whitley Council Scales. Applications and enquiries to the Director, North London Blood Transfusion Centre, Deansbrook Road, Edgware, Middlesex. (1538)

POSTDOCTORAL RESEARCH

Mass Spectrometry ion source research on ionisation of non-volatile organic and biochemical compounds from a liquid surface. Research includes instrumentation, parametric studies and applications development. Related experience in mass spectrometry, analytical instrumentation, AEI MS-9, mini-computers, and/or MS of organic or biochemical compounds considered important. Salary $11,000 to $11,500. Two-year appointment possible. Contact C. A. Evans, Jr., Materials Research Laboratory, University of Illinois, Urbana, Illinois 61801 U.S.A. (1521)

IMPERIAL COLLEGE

Postdoctoral Research Assistant in Precambrian Stratigraphy

Applications are invited for the above post which is tenable in the Geology Department under the direction of Dr M. D. Muir and is supported by the metal mining industry. The work will involve the application of geological and micropalaeontological correlation methods to mid-precambrian stratigraphy and mineral exploration.

Candidates should hold a Ph.D. or have appropriate industrial or research experience in earth or life sciences.

The post (starting salary £2,118 per annum plus £213 London allowance, with F.S.S.U.) is tenable for up to three years. Threshold payments at the authorised rate apply to this post.

Further details may be obtained from Dr M. D. Muir, Department of Geology, Royal School of Mines, Prince Consort Road, London SW7. Applications with the names of two referees should reach Dr Muir at the above address not later than November 1, 1974. (1495)

IMPERIAL COLLEGE

DEPARTMENT OF CHEMISTRY

Applications invited for S.R.C. Postdoctoral Research Assistantship with Dr W. P. Griffith on interaction of Osmium tetroxide with opel and actual biological tissue and membrane materials. Tenable for one year. Salary up to £2,460 including London allowance, plus threshold payments and with F.S.S.U. Commencing preferably on December 13, 1974. Previous experience in preparative organic or organometallic chemistry or biochemistry is desirable. Applications (with curriculum vitae and names of two referees) to Dr W. P. Griffith, Chemistry Department, Imperial College, London SW7 2AY. (1465)

UNIVERSITY COLLEGE GALWAY

DEPARTMENT OF MICROBIOLOGY

POSTGRADUATE RESEARCH ASSISTANT

Applications are invited for the post of postgraduate research assistant to participate in a Euratom sponsored project on the effects of radiation on blue-green algae. Suitable candidates should be qualified in Microbiology or a related field and may register for a higher degree. Full particulars from Dr J. A. Houghton, Department of Microbiology. (1517)

TECHNICIAN

required in the Department of Plant Biology and Microbiology to assist in research and teaching in the Microbial Genetics laboratories. Previous experience together with I.S.T. or C & G Science Laboratory Technicians Advanced Certificate, H.N.C. or similar qualification. The post will be at Grade 5 salary scale £2,007 by £75 to £2,382 p.a. but if candidate does not have the appropriate experience and qualifications appointment would be on the Grade 3 salary scale £1,650 by £54 to £1,920 p.a. In both cases in addition £228 p.a. London Weighting and current threshold payment. Five day week. Five weeks (in total) annual leave. Pension Scheme. Letters only to Assistant Secretary (Establishment) E/PB, Queen Mary College, Mile End Road, London E1 4NS, stating age, qualifications and experience. (1468)

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

(University of Reading)

A Scientific Officer is required in the Chemical Microbiology Department to work on biological problems connected with milk processing. A candidate with ambition is needed who preferably also has an interest in physical or colloid chemistry.
within the scale £1,592 to £2,675 p.a. The post

Minimum qualification is H.N.C. or equivalent. Salary according to qualifications and experience is pensionable.

Apply on forms obtainable from Secretary, N.I.R.D., Shinfield, Reading RG2 9AT. Quote reference 74/35. (1498)

Government of Malawi

Ministry of Agriculture and Natural Resources

Fisheries Officer

Salary up to £4,446 + Tax Free Gratuity

To be responsible for the control and administration of a Fisheries Area in Malawi. The work will involve the management of the fresh water fisheries development and extension programme; mechanised fishing; fish marketing; fisheries training; fish farming and boat building.

Candidates, aged 25–50, should hold a degree in Biology, Fisheries Management or related natural science subject, preferably with some postgraduate training or experience in Fisheries work. They should be able to plan and execute development programmes and be competent to handle small craft. They must be capable of controlling staff. Some administration experience would be an advantage.

Generous paid leave with free passages and baggage allowance. Education allowances and subsidised housing. Loan for the purchase of a car. Free medical attention.

Please apply to MALAWI BUYING AND TRADE AGENTS, Recruitment Section, c/o Berners Hotel, Berners Street, London W1A 3BE, for application form and further details quoting reference 793/D.

(1535)

HEAD OF PHARMACOLOGICAL DEPARTMENT

interested in CARDIO-VASCULAR research is wanted by large European pharmaceutical company for its Brussels research Centre.

Applicants should be graduate in pharmacology, preferably M.D. and member of the British Pharmacological Society. The successful applicant will have the responsibility of conducting cardiovascular research on new substances with the help of well trained technicians. Possibility to extend the responsibilities to research in general pharmacology.

Our modern and well equipped research laboratories are located in the suburbs of Brussels. Domiciliation in Brussels is required. Knowledge of French is highly desirable.

A good starting salary will be offered and reviewed regularly.

For application, please send complete résumé to: Dr F. Binon, LABAZ, 1 avenue de Béjar, 1120, Brussels, Belgium. (1520)

Opportunities for Graduate Organic Chemists

If you have a degree in Chemistry, we can offer you an opportunity to work as a member of an interdisciplinary team attacking challenging problems in pharmaceutical research. The work is concerned with the synthesis of organic compounds with novel structures for evaluation as therapeutics by our biologists.

Our laboratories at Welwyn Garden City provide excellent accommodation and facilities for innovative research and you would be encouraged to keep abreast of current developments by attending seminars and making use of our library and information services.

Excellent conditions of service and fringe benefits are offered, including a generous contributory pension scheme and, where appropriate, assistance with relocation would be available.

If you would like to know more about these posts and the Company generally, please write, quoting reference R/ART to the Welwyn Personnel Manager.

ROCHE

Roche Products Limited
Welwyn Garden City Hertfordshire AL7 3AY

(1528)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
(University of London)
COURTAULD INSTITUTE OF BIOCHEMISTRY

Clinical Biochemist (Research Assistant Grade) required in the Clinical Chemistry Laboratory. The post is suitable for a recent graduate to train in Clinical Chemistry with a view to taking the M.C.B. Salary according to age and experience within the range £1,560 to £2,265 (inclusive of London allowance) plus superannuation with F.S.S.U. Applications, including a curriculum vitae and the names and addresses of two referees to the Director, Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London W1P 5PR, by November 15, 1974. (1499)

KENNEDY INSTITUTE OF RHEUMATOLOGY

CHIEF TECHNICIAN

required for the Division of Experimental Pathology. The work involved is mainly of a research nature and histology autoradiography, and electron miscroscopy services are provided for other divisions in the Institute.

This is a well equipped, modern laboratory investigating lymphoid tissue function. All the usual techniques required to study cells and tissues are employed. A suitable candidate would be expected to have the relevant background experience as well as to be prepared to take on administrative responsibilities. Written applications giving full curriculum vitae and naming two referees to: Laboratory Superintendent, Kennedy Institute of Rheumatology, Bute Gardens, London W6 7DW. (X1507)

UNIVERSITY OF ABERDEEN

SENIOR LECTURESHIP IN MEDICAL PHYSICS

Applications are invited from experienced radioisotope hospital physicists to be responsible for team of physicists, technicians and ancillary staff engaged in wide range of research activities and providing nuclear medicine service to the Grampian Area Hospital Board. The sucecssful candidate will also be responsible for team providing D.M.R. physics course, and join in M.Sc. (Medical Physics) teaching. Salary on scale £4,707 to £5,844 with initial placing according to qualifications and experience.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by November 9, 1974. (1509)

Ministry of Agriculture, Fisheries and Food
Food Science Division, London

Food Scientists/ Chemists

■ **Advise on manufacture and composition of foods and beverages** ■ **Liaise with Government departments, industry and research organisations** ■ **Participate in current awareness studies.**
□ 1st/2nd hons degree or equivalent in food science/technology, chemistry or a related science □ 4 years' postgraduate experience in food industry or research □ Age under 32 □ Appointment as Senior Scientific Officer (over £3700 – around £5000) □ Ref: SB/11/AE.

Food Chemists/ Biochemists/ Analytical Chemists

■ **Advise on manufacture and composition of foods, particularly the functions and uses of food additives and processing aids in food manufacturing processes and their specifications and analytical determination.**
□ 1st/2nd hons degree or equivalent in chemistry or biochemistry □ 4 years' experience in food industry or research □ Knowledge of metals and other contaminates of food, together with an understanding of relationships between chemical constitution and biological activity an advantage □ Age under 32 □ Appointment as Senior Scientific Officer (around £3550 – over £4800) □ Ref: SB/12/AE.
□ Application forms (for return by 7 November 1974), from Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222 ext. 500 or, (for 24 hour answering service), London 01-839 1992.

Ministry of Agriculture, Fisheries and Food
Royal Botanic Gardens, Kew

Botanists

■ **(Ref: SB/11/AH). To work in Mycological Section on taxonomy of Ascomycetes** ■ **Assist in curating Herbarium collection** ■ **Answer routine enquiries.**
□ 1st/2nd hons degree in Botany □ Age under 32 □ Appointment as Senior Scientific Officer (over £3400 – around £4700), Higher Scientific Officer (£2700 – over £3600), or Scientific Officer (around £1850 – over £2900), depending on age, qualifications and experience.
■ **(Ref: SB/12/AH). In Herbarium and library** ■ **Scan current literature for references to be included in indexes of plant names and bibliographies** ■ **Assist in routine naming and curation in the Herbarium** ■ **Undertake taxonomic research in the Herbarium.**
□ Degree, HNC or equivalent in Botany □ Interest in literature on natural history and geography □ Age under 27 □ Appointment as Scientific Officer (around £1850 – over £2900).
□ Application forms (for return by 6 November 1974), from Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222 ext. 500 or, (for 24 hour answering service), London 01-839 1992.

Ministry of Agriculture, Fisheries and Food
Fish Diseases Laboratory, Weymouth

Pathologist/ Histopathologist

■ **Undertake research and diagnostic work into diseases of marine and freshwater fish in fish farms** ■ **Develop knowledge in little known field of biology.**
□ 1st/2nd hons degree in appropriate subject, or veterinary qualification □ Postgraduate experience in pathology and histopathology essential □ Age under 32 □ Appointment as Senior Scientific Officer (£3300 – around £4600) □ Ref: SB/18/AD.
□ Application forms (for return by 8 November 1974), from Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222 ext. 500 or, (for 24 hour answering service), London 01-839 1992.

Science group
CIVIL SERVICE

(1541)

KING'S COLLEGE HOSPITAL
MEDICAL SCHOOL
(University of London)
DENMARK HILL, LONDON SE5 8RX

Graduate required to work on the development of radioimmunoassay systems for the determination of steroids, prostaglandins and their synthetic analogues. Applications to the Secretary of the Medical School not later than November 1, 1974. (1500)

BRUNEL UNIVERSITY
PHYSICS DEPARTMENT
RESEARCH ASSISTANT

(Salary scale £1,539 to £1,983 per annum. A London Allowance of £213 is payable).

Applications are invited for the above post which is connected with a research project for the study of Thin Film Thermocouples and Heat Transfer gauges for use in aerodynamic investigations. Vacuum evaporation and sputtering techniques will be required. The appointment is initially for one year, but may be extended.

Postcard for application form and further details from the Assistant Secretary (Establishment), Brunel University, Uxbridge, Middlesex or telephone UXBRIDGE 37188 extension 49. Closing date: November 1, 1974. (1511)

UNIVERSITY OF THE
WITWATERSRAND
Johannesburg, South Africa
FACULTY OF MEDICINE AND
SCHOOL OF GENETICS
CHAIR IN HUMAN GENETICS

This is a new post in the Faculty of Medicine as well as in the School of Genetics. The Chair is not attached to a specific department, though, for administrative purposes, the appointee will be allocated to one of the Medical School Departments, in accordance with the successful applicant's background and wishes, and the needs of the University. Although this arrangement will obtain initially, it is envisaged that ultimately a separate Division of Human Genetics will be established within the existing School of Genetics. The successful applicant should be medically qualified. He will be expected to teach medical students, science and honours students majoring in subjects taught at the Medical School, and general science students at the Milner Park campus. Experience in heredity counselling will be an advantage, and the encumbent will be expected to co-ordinate existing counselling services.

Salary will be determined according to qualifications and experience and will be either R13,800 (fixed) or within the range R11,040 to R12,420.

Interested persons should obtain a copy of the information sheet relating to this post from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa with whom applications should be lodged not later than November 30, 1974.

U.K. Applicants may obtain the information sheet from the London Representative, University of the Witwatersrand, 278 High Holborn, London, W.C.1 to whom a copy of the application should be sent. (1513)

MEDICAL RESEARCH COUNCIL
Demyelinating Diseases Unit

SENIOR VIROLOGIST

To join a team working on an interdisciplinary approach to the study of multiple sclerosis and related neurological conditions. Applicants should be age 27 or over, and have wide postgraduate experience in virus "rescue" methodology and an interest in neuropathic viruses especially measles, distemper, para-influenza, herpes and visna. The appointment will be for not less than 5 years initially.

Applications with curriculum vitae, list of publications and the names of two referees, should be sent to The Director (Dr H. M. Wisniewski), MRC Demyelinating Diseases Unit, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE. (AK 1545)

Ministry of Agriculture, Fisheries and Food
Fisheries Laboratory, Burnham-on-Crouch

Chemist

■ In Marine Environment Protection Division ■ Develop and improve analytical techniques for petroleum hydrocarbons ■ Examine samples of water, sediment, fish and other marine organisms ■ Support laboratory and field experiments and monitor results.

□ Degree, HNC or equivalent in chemistry □ Experience of GLC analysis essential and of oil analysis and Mass Spectrometry an advantage □ Age under 27 □ Appointment as Scientific Officer (around £1750 – over £2800) □ Ref: SB/15/AD.

Effluent Control Officer

■ Inspect waste disposal operations ■ Liaise with industry and other bodies on waste disposal ■ Assess physical and other effects of wastes dumped at sea.

□ HND, degree or equivalent professional qualification in relevant subject □ Experience of water or effluent quality control desirable □ The appointee must be prepared to spend periods away from station and will be expected to drive □ Age under 30 □ Appointment as Higher Scientific Officer (£2600 – over £3500) □ Ref: SB/17/AD.

Ministry of Agriculture, Fisheries and Food
Fisheries Laboratories, Burnham-on-Crouch

Sedimentologist

■ In Marine Environment Protection Division ■ Examine samples of marine sediments from estuarine, coastal and offshore areas ■ Carry out sediment surveys with an ecological team ■ Develop and improve methods of sediment analysis ■ Advise on sedimentological aspects of fauna distribution, waste disposal and gravel extraction.

□ Degree, or equivalent in geology or related subject □ Interest or experience in marine sedimentology desirable □ Some biological training an advantage □ Age under 27 □ Appointment as Scientific Officer (around £1750 – over £2800) □ Ref: SB/16/AD.

□ Application forms (for return by 8 November 1974), from Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222 ext. 500 or, (for 24 hour answering service), London 01-839 1992.

Science group
CIVIL SERVICE

(1541)

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF ZOOLOGY

A position exists immediately for a TECHNICAL ASSISTANT in cell culture. Applicants should have a first degree in a biological science, H.N.C. or equivalent experience. Some experience in tissue culture would be useful. The successful applicant will take over the maintenance of tissue culture facilities and will assist in research projects. The post is funded by the Medical Research Council, Salary £1,500 to £2,163 according to age and experience.

Application forms can be obtained from Dr R. T. Johnson, Department of Zoology, Downing Street, Cambridge CB2 3EJ. (1519)

UNIVERSITY OF OXFORD
PHYSIOLOGIST *or* BIOCHEMIST

Research assistant required for the investigation of control of long- and short-term gonadal changes in mammals. Techniques will include radioimmunoassay. Project aided by a grant from the Medical Research Council. Post tenable for 3 years. Initial salary up to £2,118 p.a. Applications including curriculum vitae and names of two referees to be sent to Dr J. R. Clarke, Department of Agricultural Science, Oxford University, Parks Road, Oxford OX1 3PF. (1523)

RHODES UNIVERSITY
GRAHAMSTOWN
SOUTH AFRICA

Applications are invited for the post of

SENIOR LECTURER/LECTURER
IN PHARMACOLOGY

in the School of Pharmaceutical Science from January 1, 1975, or as soon as possible thereafter.

The salary scales are: LECTURER—R4,800x300 to R6,900 per annum; SENIOR LECTURER—R6,300x300 to R8,100 per annum (These scales are presently under review). (**Note:** £1=approximately R1.60.)

A supplement of 15% on the above scales and a vacation savings bonus are also payable. The successful applicant will become a member of the University's pension and medical aid schemes.

Preference will be given to applicants who hold a Master's degree or higher qualification in Pharmacology.

Full particulars relating to the post and staff benefits together with application forms, may be obtained from the Registrar, Rhodes University, Grahamstown 6140, South Africa, to whom completed applications with copies of recent testimonials and a photograph, should be sent by November 16, 1974. (1544)

nature

A vacancy will arise soon in the Washington office of *Nature* for the important post of Assistant Editor responsible for supervising the refereeing of biological manuscripts submitted to the Washington office. A period of training in the London office under the supervision of the biological manuscripts editor will precede the posting to Washington. The successful applicant may expect to be in Washington for not less than two years.

This job is an outstanding opportunity for a recently qualified British or American Ph.D who has a strong interest in ensuring the high quality of the biological papers we publish.

Apply with curriculum vitae and the names of two referees to:—

The Editor,
Nature,
Macmillan Journals Ltd.,
4 Little Essex Street,
London, WC2R 3LF

by November 15th

Queensland Institute of Technology

QUOTE: V.95/74

HEAD OF DEPARTMENT OF PHYSICS

The Department of Physics is one of five academic departments within the School of Applied Science. The Department is responsible for:

(i) a Bachelor of Applied Science Degree course in Physics,

(ii) Associate Diploma courses in Diagnostic and Therapeutic Radiography, and

(iii) a Master of Applied Science degree course in Medical Physics which is intended for introduction in 1975.

Service physics subjects are taught to other degree students within the School of Applied Science and to Engineering School students.

There are currently 20 full-time academic staff within the Department of Physics.

Applicants should possess appropriate qualifications in physics and have relevant experience within tertiary educational establishments. Applications would also be welcomed from persons with experience in industrial or applied research areas.

Salary: $16,389 per annum.

Further information can be obtained from the Registrar, Queensland Institute of Technology, P.O. Box 246, North Quay, 4000, with whom applications close on October 26, 1974. Applications should contain the names of three referees.

(1539)

UNIVERSITY OF THE WEST INDIES — TRINIDAD

Applications are invited for:

(a) SENIOR LECTURESHIP

or

(b) LECTURESHIP IN AGRICULTURAL ECONOMICS

in the Department of Agricultural Economics and Farm Management. Preference will be given to a person with particular interest in economic theory, and agricultural policy and development. Salary scales: (a) TT$18,108 to TT$26,730 p.a.; (b) TT$13,200 to TT$20,904 p.a. (£1 sterling=TT$4.8). F.S.S.U. Unfurnished accommodation for maximum of three years at 10% of salary; thereafter 20% of salary payable in lieu of housing. Family passages; study leave. Detailed applications (6 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail as soon as possible to the Secretary, University of the West Indies, St Augustine, Trinidad. Further particulars will be sent to all applicants. (1516)

FOURAH BAY COLLEGE UNIVERSITY OF SIERRA LEONE

Applications are invited for LECTURESHIP IN GEOGRAPHY. Preference will be given to applicants with teaching and research interest in Geomorphology. Appointee will be expected to teach both General and Honours Degree students and conduct research. Salary scale (under review): Le2,400 to Le4,740 p.a. (£1 sterling=Le2). The British Government may supplement salary in range £950 to £1,300 p.a. (sterling) for married appointee or £350 to £700 p.a. (sterling) for single appointee (normally free of all tax) and provide children's education allowances and holiday visit passages. F.S.S.U. Family passages; various allowances; regular overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, not later than November 18, 1974, to the Secretary, University of Sierra Leone, Private Mail Bag, Freetown, Sierra Leone. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. (1526)

ROYAL POSTGRADUATE MEDICAL SCHOOL

POSTDOCTORAL CHEMIST OR BIOCHEMIST

required for Department of Clinical Pharmacology to undertake studies of drug metabolism in animals and man. Applicant should be conversant with metabolite separation and identification techniques; experience in the use of gas chromatography and mass spectrometry is particularly desirable.

Post available for two years. Salary according to age and experience, on scale up to £2,781 per annum.

Applications to the Personnel Officer, R.P.M.S., Hammersmith Hospital, Du Cane Road, London W12 0HS, quoting Ref. No. 20/487/N. (1515)

UNIVERSITY OF READING

WOLFSON FOUNDATION OILSEED RESEARCH PROJECT

Applications are invited for two Research Associates to join the University's Research Group working on the breeding, agronomy and chemistry of temperate oilseed crops.

The programme is financed over a 5 year period by the Wolfson Foundation Capital, and the successful candidates will be attached either to the Department of Agriculture or of Agricultural Botany according to research and experience.

Candidates should have postgraduate experience preferably a Ph.D. in Applied Plant Genetics, plant breeding or experimental agronomy, and must have a special concern for the practical application of the research programme.

Salary in range £2,050 to £2,602 including F.S.S.U. The appointment will be initially for three years with possibility of extension to a maximum of 5 years.

Further details may be obtained from Professor Watkin Williams, Plant Science Laboratories, University of Reading, Whiteknights, Reading RG6 2AS, to whom application including the names of two referees should be sent. (1533)

ISLE OF MAN

DEPARTMENT OF MARINE BIOLOGY

Port Erin

(University of Liverpool)

SENIOR LABORATORY TECHNICIAN

Candidates will be expected to have a good knowledge of biological laboratory procedures and supervise some junior technicians. An interest in marine organisms, and a willingness to organise the collection of such material on the shore and from the research vessel would be an advantage. Candidates must possess an HNC or Final City and Guilds in an appropriate subject, and have several years of laboratory experience. Initial salary within a range up to £2,163 per annum (under review) plus threshold payments. Further particulars may be obtained by writing to Professor E. Naylor, Marine Biological Station, Port Erin, Isle of Man.

Application forms may be obtained from the Registrar, The University, P.O. Box 147, Liverpool L69 8BX. Quote ref RV/277/N. (1542)

THE NEW YORK STATE VETERINARY COLLEGE

A STATUTORY COLLEGE OF THE STATE UNIVERSITY AT CORNELL UNIVERSITY

is seeking a

CHAIRMAN OF THE DEPARTMENT OF PATHOLOGY

Applications and nominations are invited for the position of Chairman, Department of Pathology, available February 1, 1975. The applicant should have a veterinary degree with advanced training and experience in veterinary pathology. The Chairman will be responsible for the leadership of a large and diversified department. Salary and rank are negotiable and to be commensurate with training and experience. Applicants should direct their correspondence to Dr James H. Gillespie, Chairman Search Committee, New York State Veterinary College, Cornell University, Ithaca, NY 14853.

An Equal Opportunity/Affirmative Action Employer. (1543)

Senior CNS Pharmacologist

Lilly Research Centre Surrey

We seek a broadly-based Neurophysiologist or Neuropharmacologist to lead a newly created team of eight graduate and technical staff conducting fundamental research on neuroleptic and antidepressant drugs.

The Lilly Research Centre is one of the most modern and well equipped establishments of its kind in Europe, and is attractively situated in its own wooded grounds near Windlesham in Surrey.

Over 100 graduate scientists work at the Centre and major research projects are directed towards the discovery and development of potential new medicines for the treatment of allergy, inflammatory disease and disorders of the central nervous system. The CNS research programme has particular emphasis on behavioural pharmacology, CNS biochemistry and neuroelectrophysiology.

The person appointed will be one of three pharmacology team leaders and will be responsible for:

- Planning and executing a soundly-based CNS research programme.
- Promoting interdisciplinary co-operation with other research teams at the Centre and liaising with outside workers.
- Keeping abreast of new developments in his field and striving to improve the model systems and methodologies he uses.
- Leadership, guidance, encouragement and development of his own team.

He will spend the greater part of his time in active research, and will be encouraged to publish important findings.

The ideal candidate for this appointment will have at least three years' post-doctoral research experience in neurophysiology or neuropharmacology. He (or she) will be aged about 28-35, and will currently be working on an appropriate research topic in an academic or industrial environment. He should be capable of considerable further development as a research scientist and be able to bring ideas and leadership skills to the position.

The starting salary will be negotiable within wide limits, as it is our client's intention to attract a first rate research scientist. Other benefits include non-contributory superannuation, free life assurance, heavily subsidised membership of BUPA, an excellent income protection scheme, and generous relocation expenses.

Please write in complete confidence for further information and an application form, or 'phone if you would like to discuss the position:

J.A. Edwards, Talentmark Limited,
King House, 11 Westbourne Grove,
London W2 4UA. Tel: 01-229 2266.

Talentmark
Biomedical and Scientific Consultants

(1548)

PHARMACOLOGISTS

We are a major international pharmaceutical company and we are seeking enthusiastic and competent graduates in either pharmacology or a related discipline to join our Pharmacology Department. An interest in either anti-inflammatory or central nervous system pharmacology would be desirable, though not essential.

We offer salaries based on qualifications and experience commensurate with the responsibilities of the positions; conditions of employment are in keeping with the best modern practice.

Assistance with relocation expenses will be available in appropriate cases.

Organon

Write with details of age, qualifications and career to date to R. W. Pollock, Personnel Manager, Organon Laboratories Limited, Newhouse, Motherwell, Lanarkshire.

(1508)

WESTFIELD COLLEGE
(University of London)
CHEMISTRY DEPARTMENT

Applications are invited for the following posts on the Medical Research Council's Steroid Reference Collection.

JUNIOR EXPERIMENTAL OFFICER

This post offers varied and interesting work suitable for a young lady with A-level or comparable qualifications, preferably but not necessarily including chemistry. Salary within the range of £1,211 to £1,679 (under review) plus threshold, depending upon qualifications and experience.

POSTDOCTORAL or GRADUATE RESEARCH ASSISTANT

to engage in synthesis of steroids for the Collection. Much of this work involves research into new methods of synthesis, or the synthesis of novel steroids. Suitably qualified graduates may be permitted to undertake postgraduate studies for the M.Phil. or Ph.D. degree. Salaries: Postdoctoral scale £2,118 to £2,412 plus threshold and London Weighting (under review) plus threshold; Postgraduate student, equivalent to an S.R.C. studentship (under review).

Applications, including the names of two referees, should be addressed to Dr D. N. Kirk, Chemistry Department, Westfield College N, Kidderpore Avenue, Hampstead, London NW3 7ST. (1534)

UNIVERSITY OF PAPUA NEW GUINEA
(Port Moresby)

Applications are invited for the post of CHIEF CARTOGRAPHER in the Department of Geography. Applicants must be experienced Cartographers. Appointee will be responsible for the supervision of junior cartographers, and will be required to prepare maps for research, publications and classes. A knowledge of photographic and lithograhpic reproduction as well as basic photogrammetric procedures would be an advantage. The Department of Geography possesses a standard cartographical equipment plus a phototypesetter. The Chief Cartographer's office and the work laboratory are air-conditioned. The level of appointment will be determined by qualifications and experience. Salary scale: A$9,099 to AS10,432 p.a. plus A$500 p.a. dependants allowance, if married or A$200 p.a. dependants allowance if single. (These salaries include an overseas allowance). (£1 sterling=A$1.78). Contracts are usually for three years but a two-year appointment would be negotiable. Conditions include provision of housing, F.S.S.U. and leave fares. Further details about the University and the conditions of appointment may be obtained from the Secretary. Applications should be in duplicate and include particulars of age, nationality, marital status, qualifications and experience, names and addresses of three referees, a recent small photograph and an indication of estimated starting date. Applications should be sent to K. R. Long, Secretary, P.O. Box 4820, University, Papua New Guinea. Closing date: November 13, 1974. (1527)

THE UNIVERSITY OF LIVERPOOL
SUB-DEPARTMENT OF IMMUNOLOGY
RESEARCH TECHNICIAN
(Grade 5)

Required to assist with M.R.C. supported project on transplantation.

Applicants should have much laboratory experience, preferably in immunological techniques.

Apply, giving details of qualifications and experience and the names of two referees, to:

Dr John Bradley,
Director,
Sub-department of Immunology,
University of Liverpool,
1st Floor Nuffield Wing,
Medical School,
Crown Street,
Liverpool L69 3BX. (1523A)

INFORMATION SCIENTIST

Senior management in the Malaysian natural rubber industry needs to be kept informed of techno-economic and statistical trends in rubber consuming countries. An abstracts-based information bulletin has been started as the core of a new information service for management, and a computerised retrieval system is now being developed.

An information scientist is required to develop and operate these services at the Malaysian Rubber Producers' Research Association.

Please apply to:

The Director,
The Malaysian Rubber Producers' Research Association,
Brickendonbury,
Hertford SG13 8NP. (1524)

THE YOUNGER RESEARCH WORKERS INTERCHANGE SCHEME 1974

Applications are invited from British research workers in the Physical Biological and Social sciences and in all fields of technology for short visits to centres working on similar programmes in any of the following countries:- Austria, Belgium, Denmark, Finland, West Germany, The Netherlands, Norway, Poland, Spain, Sweden, Switzerland. Details and application forms have been sent to University and Polytechnic Registrars for circulation. The completed forms should be endorsed by the appropriate Head of Department and be returned to the address below by November 12, 1974: Higher Education Department, The British Council, 10 Spring Gardens, London SW1A 2BN.

(1554)

EAST BIRMINGHAM HEALTH DISTRICT

CYTOGENETICS

Cytogenetist required (salary as for top grade Biochemist, presently £5190 - £5919) to take charge of a new Regional Cytogenetics Laboratory within the East Birmingham Hospital Laboratory system. New building under construction. Candidates should have higher degree (Ph.D. or equivalent) and wide experience of Cytogenetics.

The work of the department will include elucidation of chromosome abnomalities, anti-natal diagnosis of chromosomal defects, post graduate teaching and research. The Director of the laboratory will also have duties in diagnostic Cytogenetics in the Department of Human Genetics in the Infant Development Unit at the Birmingham Maternity Hospital adjoining the University. The successful candidate may be accorded honorary status in the University of Birmingham.

Candidates are invited to visit the Laboratory by direct arrangement with the Regional Virus Laboratory (Tel: 021-772 4311 Ext. 675).

Applications (1 copy), must include the names of three referees, age and relevant experience and must be received by the District Administrator, East Birmingham Health District, 45 Bordesley Green East, Birmingham B9 5ST by 2nd November 1974.

(1549)

THE POLYTECHNIC OF CENTRAL LONDON
SCHOOL OF ENGINEERING SCIENCE

READER IN LIFE SCIENCES

£4,842 to £5,175 plus Threshold Agreement

Applications are invited for this newly established post in Life Sciences. The successful candidate will be expected to strengthen and extend existing fields of research by his expertise, his experience in supervising research students, his ability to attract externally sponsored research and his links with private industry and public agencies. Research in Life Sciences will continue to be concentrated in three areas (1) Bio-organic Studies (2) Aquatic pollution (3) Neurophysiology and behaviour.

The Life Sciences area has a major teaching commitment in the PCL's Modular BSc. Degree (C.N.A.A.) and is also actively involved in the development of a number of postgraduate courses. The Reader will be expected to contribute to project teaching and to participate in the planning of new courses.

Details and application form from The Establishment Officer, PCL, 309 Regent Street, London W1R 8AL. 01-580 2020 Ext. 212. Closing date November 4, 1974.

(1553)

FELLOWSHIPS AND STUDENTSHIPS

UNIVERSITY OF WARWICK
RESEARCH FELLOWSHIP
in
THEORETICAL PHYSICS

Applications are invited for an appointment as a Postdoctoral Research Fellow in the Theoretical Physics Group. The successful candidate will work on the theory of hopping conductivity in amorphous materials with Professor P. N. Butcher. Salary in the range £2,118 to £2,580 p.a. plus threshold payments and participation in FSSU. Applications as soon as possible to the Academic Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref. No: 10/A/74. (1496)

UNIVERSITY OF DUBLIN
Trinity College

PROTEIN CHEMIST

There is a vacancy under a contract with the E.E.C. Environmental Research Programme for a Postdoctoral Fellow to analyse the amino acid sequence in regions of the polypeptide coded by the trpA gene of *Salmonella typhimurium*. The Fellow will be part of a team designing tests to evaluate the likelihood that various micropollutants induce mutations in man. Parallel test systems are being developed in bacteria and in mice. The work is intellectually challenging and socially important. Applicants must have had experience of protein purification and amino acid sequencing. The salary is negotiable.

Applications should be sent to:

Professor Dawson,
Department of Genetics,
Trinity College, Dublin 2.

by November 2, 1974. (1403)

University of Cambridge

Department of Physical Chemistry

POST-DOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a post-doctoral fellowship in the research group of Dr. I. W. M. Smith. The successful applicant will determine the rates at which vibrational energy is transferred from HF and DF to other molecular species, in processes which are important in chemical lasers. Previous experience in gas kinetics, energy transfer, gas-lasers or flow-discharge systems would be an advantage but is not essential. The salary will depend on age and experience but is likely to be in the range £2,100 to £2,600 per annum plus F.S.S.U. Applications, naming 2 referees, or requests for further details should be sent to Dr. I. W. M. Smith, Department of Physical Chemistry, University Chemical Laboratories, Lensfield Road, Cambridge CB2 1EP. (1477)

NEWNHAM COLLEGE, CAMBRIDGE

proposes to elect to one or more

RESEARCH FELLOWSHIPS

tenable for 3 years from October 1, 1975. Applications are invited from women graduates of any University in Great Britain or the Commonwealth for research in any subject. Applications are also invited for the SARAH SMITHSON RESEARCH FELLOWSHIP which is restricted to students of Philosophy or Psychology but is open to women graduates of any University.

Unless she receives other emoluments, a Research Fellow who has not yet obtained a Ph.D. will receive £992 a year and free board and residence: or, if she chooses to be non-resident, she will receive £1,230 a year. For a Research Fellow who has already obtained a Ph.D. degree, the emoluments are increased by £157 a year. The Research Fellow may choose to be pensionable under the F.S.S.U.

Full particulars may be obtained from the Principal and the closing date for the receipt of completed applications is January 11, 1975. (1536)

GIRTON COLLEGE
CAMBRIDGE

A Clothworkers' Company Visiting Fellowship is available for a senior woman scientist for the academical year 1975/76. The stipend will be £2,500 per annum. Residence and commons are free. Scientists wishing their names to be considered should write to the Secretary to the Council, Girton College, by November 15, 1974. (1505)

LEICESTER POLYTECHNIC

SCHOOL OF CHEMISTRY

Applications are invited for:

(1) An S.R.C. postdoctoral research assistantship with experience in organic synthesis to collaborate with Dr R. H. Dahm on a project concerned with developing a novel small rechargeable battery containing solely organic constituents. A specialised knowledge of electrochemistry is not required. Post would interest organic chemists who desire to widen their field of interest. Normal S.R.C. salary and conditions apply. Post is tenable for up to 20 months.

(2) An S.R.C. CASE studentship to study ionic conduction and diffusion in a range of unusual compounds. Candidates will read for a C.N.A.A. Ph.D. The academic supervisors are Dr J. M. Pollock and Dr R. G. Linford and the project involves close collaboration with the Royal Radar Establishment at Malvern. Candidates should have either a first or an upper second class honours degree, a masters degree or a lower second degree with several years relevant industrial experience. The grant is £880 p.a. tax free; this would be higher in the case of students with dependants or with industrial experience.

Apply for further particulars and application forms to the Staffing Officer, Leicester Polytechnic, P.O. Box 143, Leicester LE1 9BH. (1472)

UNIVERSITY OF READING

NATIONAL COLLEGE OF FOOD TECHNOLOGY

Applications are invited for a 3 year S.R.C. (C.A.S.E.) Research Studentship to investigate the use of fungi in the treatment of liquid wastes arising from the processing of biological materials. The emphasis will be on developing an economically viable process and will include a study of the isolation of enzymes and an assessment of fungal mycelium as a source of protein for animal food. For part of the programme the student will work under Dr R. A. Grant, Tasman Vaccine Laboratories, Poole, Dorset. Graduates in Science or Technology with a biological background, with some knowledge of Microciology and Biochemistry preferred. Apply stating the names of two referees to Dr J. T. Worgan, N.C.F.T., St George's Avenue, Weybridge, Surrey. (Ref: MN 50). (1518)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

DEPARTMENT OF PHYSIOLOGY

Autonomic Nervous Control of the Cardiovascular System

Applications are invited from honours graduates in physiology, pharmacology or a related discipline, for a studentship for training in research methods tenable from November 1974. The holder will be eligible for registration as a Ph.D. student of the University. The value of the award and conditions of appointment will be those of M.R.C. Studentships.

Facilities are available for the postgraduate student to join a group working on several aspects of autonomic nervous control of the cardiovascular system in animals and in man. Applicants should write as soon as possible to Dr P. H. Fentem giving a brief curriculum vitae and the names and addresses of two referees, at the Department of Physiology, The Medical School, University of Nottingham, Nottingham NG7 2RD. (1531)

UNIVERSITY OF CALGARY

DEPARTMENT OF CHEMISTRY
BIOCHEMISTRY GROUP

Applications are invited for the position of

POSTDOCTORAL RESEARCH FELLOW

commencing early in January 1975. Research will be concerned with the structure-function relationship of

(1) proteolytic enzymes from Anthozoa; or

(2) extracellular enzymes produced by thermophilic fungi.

The initial appointment would be for one year with provision for a second year. Salary according to NRC regulations ($8,700 per annum).

Application with curriculum vitae and names of two referees to:

Dr K. J. Stevenson
Department of Chemistry
Biochemistry Group
University of Calgary
Calgary, Alberta, Canada
T2N 1N4.

(1540)

SENIOR NICHOLAS FELLOW

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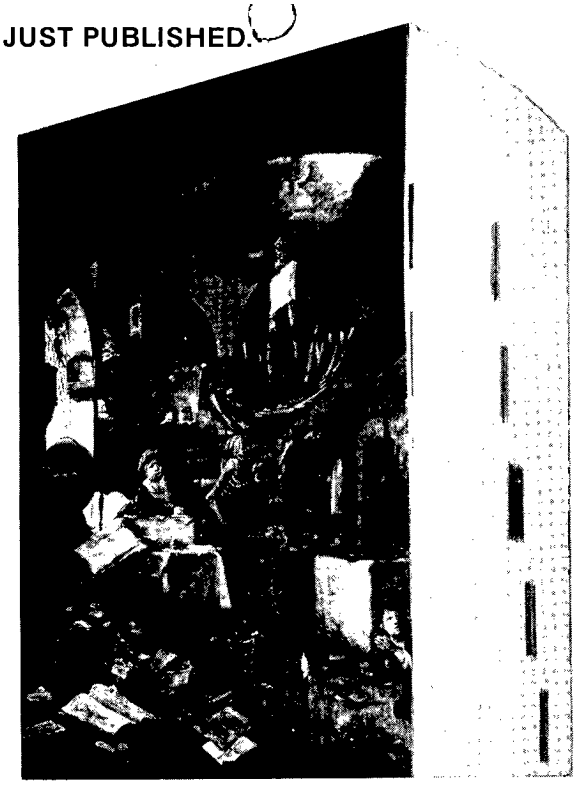

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