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
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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
Volume 252 October 18, 1974

INVESTIGATING THE PARANORMAL

For those in peril on the factory floor.

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Guide to authors
Nature accepts three types of communications:

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Characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible. Expression of preferred if 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.

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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 Personal communication and unpublished work should be incorporated in the text.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.


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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, 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 "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 was not more focused on 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 a 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 fibrosis 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? What 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.

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, 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.
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 laybooks 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 license 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 programmes 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 come 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 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 exploitation 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 of 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 Nechama 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 Morocco'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.
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, 1974; 1974); but whether they can achieve the next step—the translation of the RNA into protein—is still not known. Furthermore, this is more 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 far 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 EcoRI, 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 scalps, but no-one is yet ready even to think in concrete terms about operating on such exotic 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 10^16 labelled phage —without either immediate ill effects, or traces of survival 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 for 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
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 moated 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 a Swiss-based multinational is almost certainly based on fears that “rationa-lisation” 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.

Business report
by Roger Woodham

Major differences in both methods and aims. With his background in enzyme evolution and bacterial adaptation, Hartley expects to rely very much more heavily on 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 steatorotermophilus, 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 thermostable 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 may it is 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 outre 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 translocation 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 alpine poplar. It 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 age-long struggle”. This would appear, at first glance, contrary to Soviet utilitarian theories, but, the commentator explains, the “dialactics 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, when there were 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 striped 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 be given authority over all energy research and development, including
Fast workers

Edward 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 resynthesize 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 Aeronautics 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. Some 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 invertebrates 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 may 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.
autopsy, does not contradict the neural hypothesis as originally proposed. 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 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. This criticism would seem unfounded if the recent reports 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 are in keeping with the earlier ones. Since the full papers by Panayiotopoulos et al. and by Ballantyne and Hansen have yet to appear, it is difficult to be certain that these workers have achieved the refinements of the counting technique reported by Bradley. Scarpelesos and Panayiotopoulos 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 superposition technique (employed by ourselves) in detecting very small evoked biological signals has been accepted ever since the pioneering experiments of Dawson. 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 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 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 dystrophy is the parabiotic cross-innervation experiment of Douglas and Cosmos in mice. The strongest evidence against a myopathic aetiology (though not necessarily for a neural one) is the exceedingly elegant chimera study of Peterson. At first sight contradictory, these observations 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

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 (DVD), since expanded to include Ross Island and McMurdo Sound itself.

The original aims of the DVD 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 cool period 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 and 5); 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 high 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 differentiate 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 of their 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 Gumbolev et al have shown to use the upper few metres of sediment from Lake Vanda to trace the lake's Late Quarterly 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. Oceanogr., 7, 36; 1962) discovered that although Lake Vanda lies in a region where the mean air temperature is 

\[ -18 \, ^\circ \text{C} \]

it has a bottom water temperature of 

\[ +25 \, ^\circ \text{C} \]

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 Ragatzke and Likens (Limnol. Oceanogr., 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. now have 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 on 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 5.10 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 (ibid, 98, 340; 1956). Simple answers cannot be expected to complex questions.

In a well designed series of field experiments on carrion crows in northeast 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 hatched clutches were almost twice the size of late clutches, but the
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 nesting crows? Predation of the eggs and desertion by the adults were the main factors responsible. Krebs has pointed out that artificially 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. Crow 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 acrarin, 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 recognize 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 gradients of concentrations of small molecule(s) (Wolpert, J. theor. 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.

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 EcoRI 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) suffered two cuts. When X. laevis rDNA molecules of about 50 × 10^6 daltons were treated with excess EcoRI 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 EcoRI 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 EcoRI 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 sent 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 repeat lengths in each fragment 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 3.0 × 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 rRNA 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 EcoRI 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 histones 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 miliaris. 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, Lytechinus pictus, present in lesser amounts, 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 miliaris 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, 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 active for histone F1 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 interspersed 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, Seeiweessen) 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 Smitherman (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 inversion 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. Lirntrom (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 paleo-geographic 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.

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 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
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 germainium 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.

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 chalco-
genide 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 of 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 chalco-genide with an unusual form of surface passivation. The filament was produced by melting and drawing down a sample of chalco-genide 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 chalco-genide, 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 chalco-genides (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) 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 payoff, 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 that which 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\(^1\) 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\(^2\).

The single-source theory has been challenged by Schilling\(^3\)
on geochemical grounds, particularly with reference to Sr
isotope variation\(^4\). We propose to show that geochemical
variations in Iceland and the North Atlantic may be consis-
tent with the one-source model, both as regards petro-
chemistry and isotope geochemistry.

We have collected and analysed 182 samples that are
considered to cover volcanic activity in the tholeiitic rift
zones of Iceland\(^1\) during the last 10\(^{6}\) 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 pro-
duced 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\(^5\) (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. Alberts-
son, 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\(_2\)O content remains constant, at about 0.05-0.1%, in the Kverkfjöll area and probably along the entire adjoining ridges. A pro-
nounced positive correlation exists between K₂O, TiO₂ and P₂O₅ whereas K₂O 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 K₂O values. At a distance of 500–600 km from central Iceland the K₂O 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. 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 tholeites. 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 tholeites span almost the entire spectrum of observed compositions of oceanic tholeite. To illustrate this is a plot of MgO against iron (Fig. 3) compares the Icelandic tholeites to analyses of oceanic tholeites. In spite of this great compositional span, however, the bulk of the Icelandic analyses tend 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 tholeites are distinguished by high alkalis and iron magnesium ratios relative to oceanic basalts. The Juan de Fuca 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.

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

seems to bear an inverse relationship to lava volume. This effect could be attributed to fractional crystallisation. The Ti concentration, however, which has been shown to be sensitive to pressure, 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 tholeites, relative to low potassium tholeites which are produced at shallower depths by a higher degree
from the mantle and could indicate a higher content of Rb (phlogopite for example) in the plume than has been assumed previously.

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 volcanic-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, chiefly on the basis of Sr isotopes, has advocated two primary magma sources in the North Atlantic. Magma derived from the Icelandic plume have high (~0.7032) "Sr/"Sr ratios, 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. 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 and amphibole. 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 must contain between 1 p.p.m. and 0.1 p.p.m. Rb. The high Rb content of the phlogopite results in an increased "Sr/"Sr ratio relative to the rest of the mantle. Using the values of Rb and Sr already given for phlogopite the "Sr/"Sr ratio would increase by $7 \times 10^{-5}$ every million years. Conversely, the highest Rb/Sr ratios observed in Iceland tholites increase by $2 \times 10^{-4}$ every million years, whereas the Sr isotopic ratio of the depleted ocean tholites 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 radioisotopic dating. 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 indicates, however, that, in general, rocks from the alkaline provinces in Iceland possess slightly higher Sr isotope ratios than the tholeiites. That is consistent with their supposed nature as lower degree partial melts.

On the basis of the available data we therefore conclude that there is no well established geochemical evidence to
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 14O isotope thermometry 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. As the deuterium content of precipitation in middle and high latitudes depends mainly on the climate and decreases with annual mean temperature, the deuterium content in plants is closely related to climate, and tree rings should therefore bear information on past climates.

Sample preparation and mass spectrometric deuterium determinations have already been described. The overall measuring accuracy is ±2% and the results are reported in per mille deviation from Standard Mean Ocean Water (SMOW).

During evapotranspiration of the leaves, complicated isotope 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:

\[
\delta_i = \delta_i (1-h) + \delta_e h + \varepsilon_k (1-h),
\]

where \(\delta_i\) is the deuterium content of the leaf sap, \(\delta_e\) that of soil water, and \(\delta_k\) that of atmospheric vapour. \(\varepsilon_k\) is the equilibrium separation factor which increases with decreasing temperature, \(\varepsilon_k\) 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.

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 \(\delta_e \approx 70\%\). Because atmospheric vapour in Central Europe, contains on average, 75% less deuterium than does precipitation: \(\delta_p \approx 5\%\) (ref. 10), \(\varepsilon_p\) is temperature dependent and is \(93\%\) at \(15^\circ\)C (ref. 11), and \(\varepsilon_k (\approx 16\%)\) is independent of temperature. The relative humidity in the area around Sachsenried is about 75%, and

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<th>Table 1 Correlation coefficients between the deuterium content of <em>Picea</em> and meteorological and other parameters</th>
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* \(r_{\delta e} = 0.49\), † \(r_{\delta e} = 0.7\) (beginning in 1881).
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 2σ in Table 1.

In the 5-yr mean, there is a reasonable correlation between the deuterium content and the summer temperature \( r = 0.52; \) 2σ = 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,
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% °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; rσ = 0.33) than does the summer temperature (r = 0.04; rσ = 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 18O result from an ice core from Camp Century on the Greenland ice sheet12. There is a good relationship, although the ice reflects the isotopic composition of the annual precipitation (r = 0.61; rσ = 0.54). On the long term mean the relationship between the deuterium content again seems better with the annual temperature (r = 0.83; rσ = 0.54) than with the summer temperature (r = 0.41; rσ = 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 paleoecological 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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9 Berichte des Deutschen Wetterdienstes No. 36, 5 (Bad Kissingen, 1957).

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 organisation13. 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 hybridisation1, most of which occurs in the right arm of λ DNA, 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 ends15; (2) The P22 genetic map is circular, whereas the map of λ is linear16; (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 lyssogeny differs: P22 has two regions essential for immunity and repression whereas λ has but one11,12; (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 repressor13,14.

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

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In a parallel infection, nonlysogenic but otherwise identical \( \Phi^C \) E. coli strain was infected with \( \lambda \) c160 at 41\(^\circ\) C; no phages able to grow on E. coli lysogenic for \( \lambda \) were obtained.

**Structure of \( \lambda \text{immP22} \)**

(a) The immunity region derives from P22. Since the hybrid phages were selected for their ability to grow on a \( \lambda \) 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 \( \lambda \) parent, but like that of the P22 prophage. As expected if the ts-clear mutation affects the P22 c2 gene, E. coli lysogens carrying these hybrids as prophages are induced when shifted to 41\(^\circ\) C. (2) \( \lambda \) 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 \( \Phi^C \) E. coli strain which does not carry a P22 prophage (Table 3) (3) In other experiments, hybrids were constructed between \( \lambda \) and P22 prophages which carry amber or ts mutations in the genes near the c2 gene; in these cases, the hybrid phages inherit the amber or ts phenotype (S. Hilliker, unpublished work).

These hybrid phages—obtained from the cross of \( \lambda \text{c160} \) with P22c2ts30—are called \( \lambda \text{immP22} \) by analogy with \( \lambda \text{imm434} \) and \( \lambda \text{imm21} \), \( \lambda \) hybrids with the immunity specificity of the lambda phages 434 and 21 (refs 19 and 20).

(b) Tail genes derive from \( \lambda \). Since the hybrid phages can grow on E. coli strains, the genes determining host range must be inherited from the \( \lambda \) parent. As anticipated, the hybrid phages are as sensitive as \( \lambda \) wild type to antibodies raised against \( \lambda \) wild type phage particles. Furthermore, mutants of E. coli lacking the \( \lambda \) receptors for adsorption are also resistant to the \( \lambda \text{immP22} \) hybrids (data not shown). \( \lambda \text{immP22} \) hybrids, therefore, have tail genes from \( \lambda \).

(c) Prophage insertion specificity derives from \( \lambda \). The hybrid phages can integrate into an \( \Phi^C \) gal ara prophage and be transferred into another strain by conjugation. \( \lambda \text{immP22} \) hybrids, therefore, have the insertion specificity (attachment site and int gene) of \( \lambda \) (S. Hilliker, unpublished work).

(d) The source of other genes in the hybrids is as follows. Marker rescue tests were done by plating \( \lambda \) amber mutants on su' (nonpermissive) lysogens of \( \lambda \text{immP22} \) hybrids. For comparison, phage were also plated on lysogens of \( \lambda \text{imm434} \) (known to substitute none of the essential \( \lambda \) genes tested) and \( \lambda \text{imm21} \) (known to substitute only gene N)\(^{11} \). Recombinants with immunity of \( \lambda \) and wild type gene function were obtained.

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**Table 1**: Efficiency of plating (marker rescue experiment)

<table>
<thead>
<tr>
<th>lam</th>
<th>No prophage</th>
<th>( \lambda \text{imm434} )</th>
<th>Lysogen ( \lambda \text{imm21} )</th>
<th>( \lambda \text{immP22} )</th>
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<tr>
<td>( \lambda \text{Ban} )</td>
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<td>+</td>
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<tr>
<td>( \lambda \text{Jan6} )</td>
<td>( 2 \times 10^{-7} )</td>
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<td>3 ( \times 10^{-7} )</td>
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<td>3 ( \times 10^{-6} )</td>
<td>1 ( \times 10^{-6} )</td>
<td>1 ( \times 10^{-6} )</td>
</tr>
<tr>
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<td>2 ( \times 10^{-6} )</td>
</tr>
<tr>
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<td>1 ( \times 10^{-7} )</td>
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<td>1 ( \times 10^{-9} )</td>
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<tr>
<td>( \lambda \text{Ram5} )</td>
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<td>--</td>
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</table>

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\(^\circ\) C since \( \lambda \text{immP22} \) carries a cts allele. \( \lambda \text{imm21} \) and \( \lambda \text{imm434} \) were described by Herskowitz and Sinner\(^{11} \).
Table 2  Left-hand endpoint of nonhomology region

<table>
<thead>
<tr>
<th></th>
<th>cIII</th>
<th>N</th>
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<td>bio10</td>
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<tr>
<td>bio252</td>
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Frequency of recombinants

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<th>λimm434</th>
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<tr>
<td>λbio243</td>
<td>&lt;2×10⁻⁴</td>
<td>&lt;2×10⁻⁴</td>
<td>2×10⁻⁴</td>
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<tr>
<td>λbio10</td>
<td>2×10⁻⁴</td>
<td>4×10⁻⁴</td>
<td></td>
</tr>
<tr>
<td>λbio252</td>
<td>1×10⁻⁴</td>
<td>6×10⁻⁴</td>
<td></td>
</tr>
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</table>

Crosses were done as standard λ crosses without ultraviolet-irradiation as described by Siger and Weil. Recombinants were scored by their ability to grow on E. coli lysogenically both for P2 (which selects bio) and λ (which selects against imm). None of the parental phages grown in parallel produced progeny capable of growth on the test dilsogen at a frequency in excess of 2×10⁻⁴. In the diagram, solid line indicates λ DNA replaced.

were scored. Table 1 shows that functional alleles of mutations in λ genes O, 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 immλ recombinants in the cases of Q, S, R, B and J mutants indicates that the λimmP22 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 immP22.

Failure to find functional immλ 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 λimmP22 the immunity region of λ has been replaced by an analogous region from phage P22 (Fig. 1). The region of nonhomology (or non-compatible function) between λimmP22 and λ in the hybrid apparently includes genes N to P on the λ genetic map. The analogous region in P22 (immC 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)³⁰,⁵⁷,⁵⁸.

We have performed additional crosses to determine in detail the left-hand endpoint of the nonhomology between phages λimmP22 and λ. λbio10immP22 but not λbio243-immP22 recombinants can be formed, indicating that the region of nonhomology extends to between the endpoints of these deletions. Table 2 shows that the left-hand endpoint of nonhomology of phage λimmP22 falls within same region. Since the imm21 substitution replaces the λ N gene, these crosses provide independent evidence that the immP22 substitution also replaces λ gene N.

Functional comparisons of λ/P22 hybrids

(a) λimmP22 has the same immunity specificity as λimm21. λimmP22 hybrids establish and maintain lysogeny normally; less than 1% of the cells in a lysogenic culture are cured. These lysogens are immune to superinfesting phage of the same genotype. We tested various lambda phages for the ability to grow in λimmP22 lysogens. We also tested the ability of λimmP22 to grow in lysogens of these lambda phages. As Table 3 shows, λimmP22 grows normally in all lysogens except those carrying prophages with the immunity of phage 21 or P22. Furthermore, λimmP22 lysogens are immune to those phages carrying the immunity region of phage 21 or P22. This result implies that the immC repressor and operators of phage P22 are functionally equivalent to the repressor and operators of phage 21 and different from those of all other lambda phages tested. It should be emphasised that the immC region of P22 is sufficient to control the hybrid phage λimmP22, even though two immunity regions (imm1 and immC) control wild type P22. This result means that immC 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 λimmP22, 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 O and S. amber recombinants were obtained at frequencies between 10⁻³ and 10⁻⁴. These recombinants have less DNA than the Q⁷ parent, as judged from their ability to grow in the presence of chelating agents. Most of these phages were able to grow in λimmP22 or λimm21 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 Q⁷ region of λ. Since both phages in this region confer the specificity of late gene expression, 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 λimmP22

<table>
<thead>
<tr>
<th></th>
<th>F′lac (P22)</th>
<th>F′lac</th>
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<th>λimm21 (ba)</th>
<th>λimm434</th>
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</table>

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 10⁶. The F′lac strains are the same as those used to isolate λimmP22; the other lysogens are in strain 594. The λimm21 phages are derived from 21lacλ (ref. 20) or from λ⁷, as indicated.
Fig. 2 Electron micrographs of DNA heteroduplexes prepared by the formamide method\(^4\) from purified phage DNA. The \(b_2\) deletion serves as a position and informal calibration marker. (a) \(ab\)/\(\lambda\)immP22 heteroduplex molecule; (b) \(\lambda\)/\(\lambda\)imm21\(b_2\) heteroduplex molecule; (c) \(\lambda\)imm21\(b_2\)/\(\lambda\)immP22 heteroduplex molecule.

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

**Physical mapping of \(\lambda\)immP22**

The genetic tests presented above indicate that the \(\lambda\)immP22 chromosome is essentially a \(\lambda\) chromosome in which \(\lambda\) of which the region from the left of \(N\) to some point beyond the region of \(\lambda\) in which \(P\) has been substituted by DNA encoding the analogous functions of phage P22. In addition, \(\lambda\)immP22 and phage P22 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 \(\lambda\), \(\lambda\)imm21 and \(\lambda\)immP22. Each pair of DNS differed also by a deletion (\(b_2\), in the central region of \(\lambda\) 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 \(\lambda\)immP22 has a DNA substitution in the right arm of \(\lambda\) DNA sequences are replaced by non-homologous sequences from P22.

The regions of nonhomology between the three pairs of phages (\(\lambda\)/\(\lambda\)imm21, \(\lambda\)/\(\lambda\)immP22 and \(\lambda\)imm21/\(\lambda\)immP22) all begin at about the same point at the left (14.1—14.6 units from the \(b_2\) deletion). The \(\lambda\)imm21 and \(\lambda\)immP22 substitutions therefore have approximately the same left-hand endpoint of nonhomology with respect to \(\lambda\) DNA. Since the \(\lambda\)imm21 substitution replaces the \(\lambda\) gene of \(\lambda\), the \(\lambda\)immP22 substitution must likewise remove the \(\lambda\) gene of \(\lambda\). These conclusions confirm the results of recombinational studies shown above (Table 2).

The region of nonhomology between \(\lambda\) and \(\lambda\)imm21 is known to end to the left of \(\lambda\) genes \(O\) and \(P\) (ref. 26). The right-hand endpoint of the major region of nonhomology between \(\lambda\)immP22 and either \(\lambda\) or \(\lambda\)imm21 extends well beyond the point of nonhomology between \(\lambda\) and \(\lambda\)imm21 (Figs 2 and 3). Comparison with the physical map of Davidson and Szymbalski\(^5\) indicates that \(\lambda\)immP22 must have DNA nonhomologous with \(\lambda\) extending to the right of \(\lambda\) gene \(P\). Therefore, as suggested by earlier genetic results, \(\lambda\)immP22 has a substitution covering the \(\lambda\) DNA replication genes and origin of replication. We show below that \(\lambda\)immP22 behaves as if it has a \(P\) gene different from that of \(\lambda\).

Three small regions of nonhomology between \(\lambda\)immP22 and either \(\lambda\) or \(\lambda\)imm21 are found at the far right of the DNA heteroduplexes. The last of these is about 10.0 \(\lambda\) units from the right end of the DNA. If \(\lambda\)immP22 were formed by only two crossovers between \(\lambda\) 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 \(\lambda\) DNA in this region. Alternatively, \(\lambda\)immP22 may have been formed by multiple crossovers between \(\lambda\) and P22 DNAs, such that only the three small nonhomologous regions are from P22. We favour the view that \(\lambda\)immP22 resulted from two crossovers, since DNA–DNA hybridisation measurements\(^1\) indicate that \(\lambda\) and P22 DNAs share 18% homology in the right arm.

Whereas the heteroduplex between \(\lambda\) and \(\lambda\)immP22 shows a large region of nonhomology beginning at \(\lambda\) gene \(N\), the heteroduplex between \(\lambda\)immP22 and \(\lambda\)imm21 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 \(\epsilon_1\) region of \(\lambda\)imm21 and the \(\epsilon_2\) region of \(\lambda\)immP22 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 \(\lambda\)immP22 substitution replaces \(\lambda\) 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 \(\lambda\)immP22 hybrids grow normally in certain \(E.\) coli mutants (\(gro^P\), \(gro^N\), and \(nus\)) which fail to support \(\lambda\) growth (Table 4).

\(gro^P\) mutants of \(E.\) coli have alterations of a host replication function (\(dhaB\)) which apparently must interact with the \(\lambda\) \(P\) gene product for successful \(\lambda\) replication\(^7\). Since \(\lambda\)immP22 grows in these mutants, the P22 gene product analogous to \(P\) gene product either interacts with \(dhaB\) in a different manner or perhaps not at all. It should be noted that \(gro^P\) and \(gro^P\) 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 \(\lambda\) DNA. The locations of the \(\lambda\) genes are taken from Davidson and Szymbalski\(^5\).](image-url)
Table 4 Efficiency of plating on bacterial mutants unable to support growth of λ

<table>
<thead>
<tr>
<th>Host</th>
<th>λ+</th>
<th>λimmP22</th>
<th>λimm21</th>
</tr>
</thead>
<tbody>
<tr>
<td>groP</td>
<td>(1)</td>
<td>(1)</td>
<td>(1)</td>
</tr>
<tr>
<td>groP,A1 (dnaB)</td>
<td>2 x 10^{-6}</td>
<td>1.1</td>
<td>5 x 10^{-6}</td>
</tr>
<tr>
<td>groP A1 (dnaB)</td>
<td>2 x 10^{-6}</td>
<td>0.7</td>
<td>3 x 10^{-6}</td>
</tr>
<tr>
<td>groP A15 (dnaB)</td>
<td>2 x 10^{-6}</td>
<td>0.07</td>
<td>3 x 10^{-6}</td>
</tr>
<tr>
<td>groN,A55</td>
<td>2 x 10^{-6}</td>
<td>1.1</td>
<td>0.9</td>
</tr>
<tr>
<td>mus+4 (42°C)</td>
<td>2 x 10^{-6}</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>mus (42°C)</td>
<td>(1)</td>
<td>(1)</td>
<td>(1)</td>
</tr>
<tr>
<td>mus (30°C)</td>
<td>1.0</td>
<td>0.7</td>
<td>0.7</td>
</tr>
</tbody>
</table>

The groP and groN mutants are described in refs 27 and 29. mus strains are described by Friedman 30. Figures in parentheses indicate normalisation to this value. All strains are nearly isogenic with either gro+ or mus+. Two independent λimmP22 hybrids were tested.

dnaB gene29, do not affect growth of λimmP22, whereas the groP,A1 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 groN or mus block early λ transcription, probably by affecting the function of the early gene positive regulator, N protein. λimm21 is able to grow in these bacteria (Table 4); λimmP22, likewise, grows normally in 609 and mus' 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 phase-specific or bacterial recombination rather than "illegitimate recombination" because of the relatively high frequency with which they are formed. Not only are viable hybrids formed, but the immunity specificity of P22 and phase 21 are identical, both in function and, to the resolution afforded by heteroduplex mapping, in nucleotide sequence. Similarly, the φ 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 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 non-identical 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.

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 map31-35. 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 36. 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 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. Youngusband 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.

We thank Sandra Hilliker and Penny Toothman for allowing us to use unpublished results, Bik Kwoon Tye for initiating work on the heteroduplex, David Friedman and Bill Dove for phage and bacterial strains and Van Jarvik for technical assistance. D. B. holds a career development award from the National Institutes of Health. This work was supported in part by grants from the National Institutes of Health. D. B. is also partially supported by a grant from the American Cancer Society.

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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.\(^{5}\) 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\(^{6}\).

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.\(^{7}\) (\(B-V = +0.81\), \(U-B = -0.30\)) 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\(^{8}\). Neckel was unable to deduce any relationship between \(A_V\) and distance within a region centred on \(l = 73^\circ\), \(b = 0^\circ\), 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

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**Fig. 1** Two-colour plot for reddened O stars in Cygnus from Johnson and Morgan\(^{7}\). •, unreddened main sequence stars (from types B1 to K3); ○, O stars in the Cygnus Rift Region; ×, HDE226868, from the colours of Bregman et al.\(^{7}\).
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$^*$ 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$^*$ 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 $A_r \sim 5$ 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 $\pm 16\%$ 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 in a Friedmann universe, or it may be an instance of a collapsing object reversing implosion to explosion. For the latter poss-

sibility unconventional equations of state$^*$ or negative energy fields$^*$ are required if the theoretical framework is that of general relativity. 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 $(r, \theta, \Phi, t)$ in terms of which the line element within the object is given by

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

(1)

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

$$2GM = ar_b c^2$$

(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

$$S' = ac^2 (1 - S)$$

(3)

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

$$t = t_0 = \pi/(2c\sqrt{a})$$

(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

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

(5)

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

To calculate the spectrum received at $R_1$, define

$$S = sin^2 \theta, 0 < \theta < \pi/2$$

(6)

Then $\theta$ as a function of comoving time coordinate $t$ is given by

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

(7)

The white hole bursts out of the Schwarzschild radius at $t = t_0, \theta = \theta c$, where

$$sin \theta c = (ar_b c)^\nu$$

(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

$$dT/dt = sin \theta/sin(\theta + \theta c)$$

(9)
So an electromagnetic wave of frequency $v_e$ emitted from the surface appears to the receiver to have the frequency

$$v = v_e \frac{\sin(\theta + \theta_a)}{\sin\theta}$$  (10)

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

$$dt = \left(4\pi v_e^2 \sin\theta \pi \right) dv / \left(\pi \left(v^2 + v_e^2 - 2vv_e\cos\theta\right)^2\right)$$  (11)

Writing $E = hv$, $E_e = hv_e$, the number of photons in the range $[E, E+\delta E]$ received from the white hole per unit area at $R = R_0$ is given by

$$N(E)dE = \frac{L(t_0 - 2R_0^2)}{\pi^2 R_0^2 E} \sin\theta \pi dE (E^2 + E_e^2 - 2E_0\cos\theta)^2$$  (12)

For $E \gg E_0$

$$N(E) \propto E^{-3}$$  (13)

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

$$I(E) = EN(E) \propto E^{-3}$$  (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\(^3\) 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\(^4, 5\) 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 $\sim 10^{36}$ erg s$^{-1}$ through the dust grain heating mechanism\(^6\).

(ii) The transient nature of X-ray and gamma-ray bursts\(^7\) 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 $\mu$m

We report the successful flight of an experiment designed to measure the spectrum of the cosmic background radiation from $\sim 3$ 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\(^8\) and will be discussed in detail in a future paper; we present here the results of preliminary analysis of the flight data.

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Fig. 1 Cosmic background spectrum at 1 cm$^{-1}$ resolution:
- - - - 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 polarizing Michelson interferometer\(^4\) 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 polyethylene; 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 a 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 ~2\(^{1/3}\) higher than the 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.

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 that solar activity may be controlled by the tidal influence of the planets. In the course of a detailed study\(^5\) 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 2 h of 1100 LT were designated International Quiet Days (IQD).

\(\text{Fig. 1} \quad \text{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 \(-10 \text{ ordinate scale, indicated on the right-hand side.}}\)
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 ×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.

Fig. 2 Average percentage occurrence of AQDs (R) as a function of average sunspot number (n) 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).

The 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 \(^8\) 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 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. 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 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 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.

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 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.

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 Earth and Sun 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. It has also been proposed 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, and exhibits a particularly stable structure at sunspot minimum.

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 and Lamb 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 variations 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, 3, 2, 3, 2, 1, 1, 0 yr. It is noteworthy that, in a reported correlation between atmospheric ozone content and sunspot values, 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. 2a). A similar relationship appears for the trough values (Fig. 2b). 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 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. 2a holds, then the predicted value of 110 for the next sunspot number peak2 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. 2a, Rainfall cycle peak values against corresponding sunspot cycle peak values. For a as for b but for corresponding trough values.]

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

An intensive study of the Indian monsoon rains, 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 correlations 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 aeisic ridges. Observations of phase velocities against epicentral distances for Tonga-Samoa earthquakes 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

\[
V_z = -d(p/\rho) \left( \frac{a^3}{4\eta} \right)
\]

(1)

where \( a \) is the radius of the plume, the flow is along \( z \) axis and \( \eta \) is the coefficient of viscosity.

The volume flux is \( (\pi a^2/2) V_z \).

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 fluctuation:

\[
\delta T = -\eta V_z^2/4K
\]

(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: \( \eta = 3 \times 10^{21} \text{ g cm}^{-1} \text{ s}^{-1} \); \( K = 10^{-5} \text{ calorie cm}^{-1} \text{ cm}^{-1} \text{ s}^{-1} \); and the plume radius, \( a = 75 \text{ km} \). The most frequently quoted vertical velocities of plume flow are \( 2 \text{ m yr}^{-1} \). 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 \( 2 \text{ m yr}^{-1} \) each plume would bring up about \( 18 \text{ km}^2 \text{ yr}^{-1} \) of mantle material. Twenty plumes would bring up a total of \( 360 \text{ km}^2 \text{ yr}^{-1} \). That would be the total flux of the material moving away from the rise crests. Lithosphere is created at a rate of about \( 170 \text{ km}^2 \text{ yr}^{-1} \), 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. The drag can be increased by increasing either the number of plumes, or their vertical flow speeds or dimensions.

The, however, the flow speed is reduced to, for instance, \( 3 \text{ cm yr}^{-1} \), temperatures would become reasonable. But the total flux from 20 plumes would be reduced to about \( 6 \text{ km}^2 \text{ yr}^{-1} \). 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).](image-url)
Table 1 Typical temperature differences and volume flux for a range of flow velocities

<table>
<thead>
<tr>
<th>Flow velocity (cm yr⁻¹)</th>
<th>Temperature difference (°C)</th>
<th>Volume flux per plume (km³ yr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>6.75 × 10⁴</td>
<td>18</td>
</tr>
<tr>
<td>100</td>
<td>1.69 × 10⁴</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>1.69 × 10⁴</td>
<td>9 × 10⁻¹</td>
</tr>
<tr>
<td>3</td>
<td>~ 15</td>
<td>2.7 × 10⁻¹</td>
</tr>
<tr>
<td>1</td>
<td>~ 2</td>
<td>9 × 10⁻²</td>
</tr>
</tbody>
</table>

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, ∆T, 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 contended² that the viscous drag exerted on the bottom of the lithosphere by a typical plume, will result in the doming of the 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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1 Wilson, J. T., Phil. Trans. R. Soc., A258, 145-167 (1965).

A-type doubling in the CH molecule

Almost a year ago we set out to calculate an accurate value of the A-doubling in the lowest rotational level of the X'[2Π] 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 experiments², but its significance is reduced by the astronomical observation of the species, which was reported during the course of this work³. 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 has shown that the A-doubling of the rotational levels of a [1Π] state may be represented as a perturbation by [1Σ] states. To second order, the splitting may be expressed in terms of two parameters, (p+q) and q. For the lowest level of a regular [1Π] state, for which J = Ω = q, the doubling is simply 2(p+q), where

\[ p = 4 \sum \frac{\langle \Pi | H_{x \alpha} | \Sigma \rangle \langle \Pi | B (L^2 + L^2) | \Sigma \rangle}{E_{\Pi} - E_{\Sigma}} \]

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

where the summation extends over all vibrational levels of all [2Σ] states, the contribution from [2Σ] being taken as negative. Matrix elements are taken over both electronic and vibrational wave functions. \( H_{x \alpha} \) is the spin-orbit coupling operator, and \( B \) is the operator \( \hbar^2 \alpha^2 \pi^3 \).

An ab initio calculation of the parameters \( p \) and \( q \) was performed for CH by Hinkle et al.⁴ 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'[2Π] state by Cade and Huo⁵ were used; the inviable 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 \( B \Sigma - \Sigma \Sigma \) states were considered. The results are shown in Table 1; they are some 20%, too low.

In the present work, the calculation of Hinkle et al. was modified in several ways. Herzberg and Johns⁶ have shown experimentally that both [2Σ] 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.,⁷ 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 Hinkle et al. to produce the result labelled (a) in Table 1.

Table 1 A-type doubling in the X'[2Π] (ν = 0, J = 1) state of CH

| Hinkle et al.⁴ | 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 experiment¹ | 0.0563 | 0.0383 | 3.374 ± 0.01 |
| Astronomical experiment³ | 0.0556 | — | 3.335 ± 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 [2Σ] states from the basis set of Cade and Huo, using the ALCHEMY program⁸. Electronic matrix elements were then calculated using a program written by J. A. Hall⁹. The values now obtained for the A-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'[2Π] and B'[2Σ] states, while eight were used for the C'[2Σ] 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 $\Lambda$-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: 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 polycrylamides, 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

$$100 \left( \frac{N_p - N_w}{N_w} \right)$$

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 polycrystalline 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 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 channel in excitable membranes, we decided to examine comparative power spectra.

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 hourglass 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 10⁻⁵ 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 10⁻⁴ s to 10⁻¹ 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, 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 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 slit (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. 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; 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. 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, following Heiden, 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, t, amplitude, k, and shape y(t, k). In addition, characterising
each pulse is the interval $\varphi$ 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

$$I(t) = h_1 \delta(t - \tau_1) + h_2 \delta(t - \varphi_1 - \tau_1) + \ldots$$

For those sequences in which $\varphi_1$, $\tau_1$, and $h$ are functionally independent of one another, the power spectrum is

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

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 $y(\tau)$, and

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

For convenience, we can write $S(f) = N A(f) P(f)$ where $A(f) = \langle h^2 \rangle \langle |F(f,\tau)|^2 \rangle^2$ 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, $v(\tau)$.

In general,

$$F(f,\tau) = \int_0^\infty y(\tau) \exp(-2\pi i f \tau) d\tau$$

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

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

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

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

$$\langle F(f,\tau) \rangle \approx \int_0^{\tau_m} F(f,\tau) v(\tau) d\tau,$$

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.

We have

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

Physically there exists a maximum $\varphi$, say $\varphi_m$, beyond which $\mu(\varphi)$ is arbitrarily small. Hence, for low enough frequencies, $(2\pi f \varphi_m \ll 1)$, $\psi$ is frequency independent and, therefore, 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 proving 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 $\tau_m$ and $\varphi_m$. 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 $(2\pi f \varphi_m \ll \text{the smaller of } \tau_m^{-1} \text{ or } \varphi_m^{-1})$ 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, $\varphi = \text{constant} \tau$ or $h = \text{constant} \varphi$) exist between the pulse parameters. We note for example that $\varphi = \text{constant} \tau$ is a kind of inhibition where the presence of a pulse with a long time constant tends to delay the appearance of the next pulse while $h = \text{constant} \varphi$ 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) Peschel 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 1/2 portion of the spectrum is due to the non-Poisson character (clustering) of the individual particles. At higher
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 (Everett 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..." 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 has been criticised by Legon. Unfortunately the revised article containing the basis for some of my statements has only recently appeared though it was submitted long before the other. This paper 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 (page 32) when I wrote my paper. 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 can surely be in little doubt that I understand at least as well as does Legon the fundamental conclusion of Carnot 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 (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 artefact, 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 (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.

Failure to apply to isolated systems. I did not in fact claim 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. 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 $\Delta S_{\text{sys}}$ is always a single-valued function of the work wasted. If one part of the system is much larger than the other, the equations approach as a limit the simple form given in my paper (ref. 5, equation (10)).

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

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 irreversible 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 T₀ of the environment is equal to the temperature T of the system throughout the process". On what grounds are these doubts based? Legon does not discuss, let alone dismiss, any of the sources quoted in my article. Other relevant sources which should be considered are Keenan and Hatzopoulos and the classic accounts by Maxwell and by Gouy.

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 (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 \(w_{\text{max}}\) under isothermal conditions, it cannot conveniently be used to determine \(w_{\text{max}}\) under nonisothermal conditions because the term \(\delta T\) 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 \(w_{\text{max}}\) 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 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 graphic 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, 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.

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Table 1 Remote perception of graphic material

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

*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. 2c), 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 \( P = (10)^{11} = 3 \times 10^{-7} \).

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 x 4 x 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 2's, one 4, two 5's, and two 6's. The probability of this occurring by chance is approximately one in 10^4.

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 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...
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 assigning 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. 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 section 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 (Oz), referenced to linked mastoids, was amplified with a Grass 5P-1 preamplifier and associated driver amplifier with a bandwidth 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

<table>
<thead>
<tr>
<th>Flash Frequency</th>
<th>0</th>
<th>6</th>
<th>16</th>
<th>0</th>
<th>6</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sender</td>
<td>Average Power</td>
<td>Peak Power</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J.L.</td>
<td>94.8</td>
<td>84.1</td>
<td>76.8</td>
<td>357.7</td>
<td>329.2</td>
<td>289.6</td>
</tr>
<tr>
<td>R.T.</td>
<td>41.3</td>
<td>54.5</td>
<td>37.0</td>
<td>160.7</td>
<td>161.0</td>
<td>125.0</td>
</tr>
<tr>
<td>No sender (subject informed)</td>
<td>25.1</td>
<td>35.7</td>
<td>28.2</td>
<td>87.5</td>
<td>95.7</td>
<td>81.7</td>
</tr>
<tr>
<td>J.L.</td>
<td>54.2</td>
<td>53.3</td>
<td>44.8</td>
<td>191.4</td>
<td>170.5</td>
<td>149.3</td>
</tr>
<tr>
<td>R.T.</td>
<td>65.8</td>
<td>50.9</td>
<td>32.8</td>
<td>240.6</td>
<td>178.0</td>
<td>104.6</td>
</tr>
<tr>
<td>No sender (subject not informed)</td>
<td>39.8</td>
<td>24.9</td>
<td>30.3</td>
<td>145.2</td>
<td>74.2</td>
<td>122.1</td>
</tr>
<tr>
<td>Averages</td>
<td>65.8</td>
<td>49.9</td>
<td>43.1</td>
<td>214.5</td>
<td>169.8</td>
<td>153.5</td>
</tr>
</tbody>
</table>

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 approximation to the nonparametric randomisation test 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 to which stimulus was generated. They made their guesses known to the experimenter via one-way telecommunication. An analysis of these guesses has shown them to be at chance, indicating the absence of any supraliminal cueing, so arousals 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 exist, 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.

This research was sponsored by The Foundation for Parapsychology Research, New York City. We thank Mrs Judith Skutch, Dr Edgar D. Mitchell of the Institute of Nuetic Sciences—as well as our SKI associates, Mr Bonnar Cox, Mr Earle Jones and Dr Dean Brown—for support and encouragement. Constructive suggestions by Mrs Jean Mayo, Dr Charles Tart, University of California, and Dr Robert Ornstein and Dr David Galin of the Langley Porter Neuropsychiatric Institute are acknowledged.

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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 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.

Prominent among such models is the complex system with random interactions, studied in various forms by Gardner and Ashby and May; 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 species. That is, the number $N_i$ in the $i$th species obeys

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

All birth rates $b_i$ were taken as 1, and the self-regulating coefficients $a_{ii}$ as $-1$. The feasibility requirement was that the
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 $S_{ji} = N^*_j$, $a_{ij}$; $S$ is unstable if it has an eigenvalue with positive real part. The interaction coefficients were chosen to have a fixed magnitude $\beta$ 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 $T$.

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 and analytical), in showing that both feasibility and stability are rare when $z > T$. 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 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 $N_i^*$.

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 found1 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 $-180^\circ$ to $+180^\circ$, 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 $360^\circ$ 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 $\chi^2$ 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 χ² 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 previously⁴, and similarly to each of the records from these 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 else¹. 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 object⁶.

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 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

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**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 symmetrically 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.
Microbial activation of prophenoxiloxidase 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. An important part of the host-parasite relationship is the defence system of the host. In insects, phenoxiloxidase (o-diphenol: O₂ oxidoreductase; EC 1.10.3.1) has been suggested to be at least partially responsible for insect immunity and parasite resistance.

The enzyme produces quinones which can react with proteins and with the thiol and amino groups of other compounds. Quinones can be bactericidal. When quinones are present in excess, the black pigment melanin is formed. 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. Melanin deposition is often associated with insects’ cellular response, but can also be a strictly humoral response. In some cases of unsuccessful malaria transmission by mosquitoes there is an apparent melanisation manifest in Ross black spores and degenerate oocysts. For quinones to be involved in resistance to an insect parasite, however, control of phenoxiloxidase activity would be essential. Quinones randomly reacting with the insect host would be ineffective as antiparasitic agents. Furthermore, large amounts of active phenoxiloxidase can kill insects. As a contribution to this problem, I describe here the activation of prophenoxiloxidase in the plasma of immune Gallus gallus larvae by microbial products.

For immunisation, larvae (each larger than 200 mg) were injected with 1.0-μg doses of Shiella flexneri lipopoly-

Saccharide B (Difco) and incubated at 23° C. Control larvae were un.injected. Haemolymph was collected 18-24 h after immunisation and pooled in a CO₂ environment; at

would account for birds preferring the unfamiliar object only in the early stages of imprinting.

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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.

My preliminary experiments and those of Evans 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 650g 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 dilution, to be 18,590 mm−3 for immune and 40,040 mm−3 for control reconstituted haemolymph, slightly higher than those reported by Stephenson 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 KPO₄, pH 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’-carboxy-1’-pyrrolidinyl)-5-methyl-o-benzoquinone, with a λmax at 520 nm (ref. 7). In the system used, with excess substrates, ΔA₂₅⁰ was directly proportional to enzymatic activity. One phenoloxidase unit was defined as ΔA₂₅⁰ = +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 phenoloxidase activation than control plasmas when both were incubated with either zymosan (a yeast polysaccharide) or a preparation of damaged Pseudomonas aeruginosa (Fig. 1). After treatment with a chymotrypsin (EC 3.4.14.5) both control and immune plasmas showed a high degree of

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**Table 1** Enzyme activity in (NH₄)SO₄ fractions of immune plasma

<table>
<thead>
<tr>
<th>Protein</th>
<th>Zymosan activation</th>
<th>Phenoloxidase†</th>
<th>α-Chymotrypsin activation</th>
<th>Lysozyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>(NH₄)SO₄ fraction*</td>
<td>mg g⁻¹ lyophilisate†</td>
<td>U g⁻¹ lyophilisate</td>
<td>Specific activity§</td>
<td>U g⁻¹ lyophilisate</td>
</tr>
<tr>
<td>0-40% ppt., fraction A</td>
<td>10</td>
<td>0</td>
<td>43,950</td>
<td>4,395</td>
</tr>
<tr>
<td>40-50% ppt.</td>
<td>85</td>
<td>0</td>
<td>21,850</td>
<td>257</td>
</tr>
<tr>
<td>50-60% ppt.</td>
<td>140</td>
<td>0</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>60% Supernatant, fraction B</td>
<td>66</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Whole plasma††</td>
<td>538</td>
<td>144,045</td>
<td>258</td>
<td>153,745</td>
</tr>
</tbody>
</table>

* Plasma was prepared from reconstituted haemolymph as described in the text. Fractions were prepared by the addition of solid salt (100% saturation = 70.6 g per 100 ml). Precipitates were dissolved in 0.01 M KPO₄, pH 6.0 (the pH 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 at pH 9.6 of Laughlin buffer.
‡ 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.
‖ ΔA₂₅⁰ = −0.001 per min at 30°C of a suspension of Micrococcus lysodeikticus was one lysozyme unit.
†† No salt added, no dialysis.
prophenoxidase 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 pH 6.0 (the pH of the plasmas) or at pH 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-
\( \mu m \) 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 HSO\(_4\) 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\(^7\).

Immune plasma was fractionated with (NH\(_4\))\(_2\)SO\(_4\) (Table 1). The material precipitated with 40% saturation (fraction A) contained most of the prophenoxidase. The material still in solution after 60% saturation (fraction B) had most of the lysozyme activity. Prophenoxidase in fraction A was activated by \( \alpha \) chymotrypsin, but not by zymosan alone; however, the prophenoxidase 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\(_{m}\) of phenoxidase activated by zymosan and \( \alpha \) chymotrypsin for 4-methylcatechol were similar, 1.20 and 1.07 mM respectively. Figure 3 shows that when prophenoxidase 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.

Activation of prophenoxidases is associated with the aggregation of enzyme subunits\(^14\). 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 prophenoxidase subunits. This would explain the differences observed between control and immune plasmas in Fig. 1, since immune haemolymph contains larger concentrations of lysozyme\(^16\). Another hypothesis is that zymosan activates a proteolytic enzyme in fraction B, as it does in complement\(^1\). The proteolytic enzyme would then activate the prophenoxidase.

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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 antigens14 and, in man, greatly elevated levels of total IgE occur in most individuals infected with any one of a variety of helminths including Ascaris lumbricoides1, Capillaria philippinensis2 and ancylostomiasis3.

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 non-specific potentiation of unrelated IgE responses to such antigens as egg albumen, keyhole limpet haemocyanin, and house dust4. Present evidence indicates that live worms produce a factor which, acting via T cells5, 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 potentiated6 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 immuno- cytomas10,11 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 Rowe12.

The specific immunological reagents were prepared as follows: The purification of rat IgE from the serum or rats bearing IgE secreting immuno cytomas was as previously described14 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 equilibrated with 63 mM sodium barbiturate, 15 mM Barbitol, 1.8 mM calcium lactate buffer, adjusted to pH 8.6 with HCI. 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 (R2 monoclonal protein) emulsified in complete Freund’s adjuvant. Antisera were absorbed with a solid immunoabsorbant15 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)16 in complete Freund’s adjuvant. The antiserum was also purified by the caprylic acid technique and the resultant IgG fraction was labelled with 131I by the method of McConahey and Dixon17.

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>
<thead>
<tr>
<th>Group</th>
<th>Treatment of rats</th>
<th>Egg albumen (μg ml−1)</th>
<th>N. brasiliensis titre</th>
<th>Total serum IgE (μg ml−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal rats no treatment</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>Primary response to 1 μg EA</td>
<td>92 ± 36</td>
<td>—</td>
<td>1.33 ± 0.25</td>
</tr>
<tr>
<td>3</td>
<td>Secondary response to 10 μg EA</td>
<td>1,228 ± 231</td>
<td>—</td>
<td>1.80 ± 0.39</td>
</tr>
<tr>
<td>4a</td>
<td>Potentiated reagin response to EA 12 d after N. brasiliensis</td>
<td>1,316 ± 270</td>
<td>—</td>
<td>288 ± 47</td>
</tr>
<tr>
<td>4b</td>
<td>Not potentiated reagin response to EA 12 d after N. brasiliensis</td>
<td>4,096</td>
<td>—</td>
<td>493</td>
</tr>
<tr>
<td>5</td>
<td>Unimmunised rats 12 d after N. brasiliensis infection</td>
<td>—</td>
<td>640 ± 109</td>
<td>247 ± 42</td>
</tr>
<tr>
<td>6</td>
<td>Unimmunised rats 18 d after N. brasiliensis infection</td>
<td>—</td>
<td>—</td>
<td>104 ± 8</td>
</tr>
</tbody>
</table>

*To estimate specific reaginic antibodies by the passive cutaneous anaphylaxis technique17, 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 μg 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 vasodilatation. 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.

†The method of culture of N. brasiliensis and preparation of the infective dose have been previously described18,19.
immunised with 1 μg egg albumen (Sigma grade V) injected intraperitoneally together with 10^8 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.4-6

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 of 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.12 It is interesting that this facility is also reflected in very high levels of total serum IgE (up to 493 μg ml^-1) following helmhnt infection in rats of this strain.

The main points which emerge from these results are that infection of the rat with helmhnt 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.

This work was supported by the Medical Research Council and the Fonds Cancerologique of the Caisse Generale d’Epargne et de Retraite (Brussels). E. J. is in receipt of a fellowship from the Asthma Research Council. H.B. is a staff member of Euratom Biology Division. We thank David Haig and Jenny Naze-de-Mets, Bernadette Plateau and Jean Hiére Kints for their assistance.

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Alternative route for nitrogen assimilation in higher plants

It is generally considered that glutamate dehydrogenase (EC1.4.1.3) is the enzyme that is chiefly responsible for the incorporation of nitrogen into the α-amino group of amino acids:

\[ \alpha\text{-oxoglutarate} + \text{NH}_3 + \text{NAD(P)^{+}H^+} \rightarrow \text{L-glutamate} + \text{H}_2\text{O} + \text{NAD(P)}^\cdot \]

(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 and, although the chloroplast contains glutamate dehydrogenase, the level of activity is low and the enzyme has a high K_m for ammonia, 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. If glutamine synthetase is the route for entry for ammonia, how-
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-glutamate: α-oxoglutarate aminotransferase (NADPH-oxidising) (equation (2)), has been shown to be present in bacteria and yeasts, particularly when grown under limiting concentrations of ammonia, and in nitrogen-fixing bacteria and the bacteroids of nodulated higher plants.

\[ L\text{-glutamate} + α\text{-oxoglutarate} + NADPH + H^+ \rightarrow 2 L\text{-glutamate} + NADP^+ \] (2)

This pyridine nucleotide-linked enzyme has not been reported in the tissues of higher plants in spite of attempts by ourselves and others 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 Wolk. In a medium containing 0.33 M sorbitol, 1 mM MgCl₂, 2 mM EDTA, 1 mM mercaptoethanol, 10 mM sodium pyrophosphate, pH 7.0. The chloroplast pellet was washed in 0.33 M Sorbitol, 5 mM KCl, 10 mM sodium pyrophosphate, pH 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% (v/w) phenol, in the presence of ammonia vapour, and quantified by the method of Atfield and Morris.

The results (Table 1) show that chloroplasts are capable of converting α-oxoglutarate to glutamate in the light, confirming the findings of earlier workers. 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, pH 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.

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

<table>
<thead>
<tr>
<th>Nitrogen donor</th>
<th>Acceptor</th>
<th>Glutamate formed (μmol per mg chlorophyll h⁻¹)</th>
<th>Glutamine lost</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>α-oxoglutarate</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Glutamine</td>
<td>α-oxoglutarate</td>
<td>6.9</td>
<td>3.6</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>α-oxoglutarate</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Asparagine</td>
<td>α-oxoglutarate</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Aspartate</td>
<td>α-oxoglutarate</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>Glutamine</td>
<td>None</td>
<td>0.7</td>
<td>0</td>
</tr>
</tbody>
</table>

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, pH 7.5.

### Table 2. Glutamate formation by chloroplast extracts under various conditions

<table>
<thead>
<tr>
<th>Electron donor</th>
<th>Nitrogen donor</th>
<th>Acceptor</th>
<th>Glutamate formed (μmol per mg chlorophyll h⁻¹)</th>
<th>Glutamine lost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fd + DIT</td>
<td>Glutamine</td>
<td>α-oxoglutarate</td>
<td>23.2</td>
<td>10.8</td>
</tr>
<tr>
<td>Fd + DIT</td>
<td>NH₄Cl</td>
<td>α-oxoglutarate</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Fd + DIT</td>
<td>Asparagine</td>
<td>α-oxoglutarate</td>
<td>2.08</td>
<td></td>
</tr>
<tr>
<td>Fd + DIT</td>
<td>Aspartate</td>
<td>α-oxoglutarate</td>
<td>1.55</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>Aspartate</td>
<td>None</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Fd + DIT</td>
<td>Glutamine</td>
<td>None</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

The reaction mixture consisted of 70 μmol tricine, pH 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 μg, sodium dithionite + NaHCO₃ (DIT) 1.6 μg each, FMN 0.2 mg, methyl viologen (MV) 0.1 mg, NADPH 0.4 mg. Amino donors were present at 2 mM concentration except asparagine which was at 5 mM. Ferredoxin was derived from *Spirulina maxima*.

*No visible glutamate spot on the chromatogram.
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. The nitrate is metabolised through nitrate and nitrite reductases to ammonia. Both of these reactions are strongly light stimulated, as is the incorporation of $^{15}$NO$_3$, $^{15}$NO$_2$, and $^{15}$NH$_3$ into $\alpha$-amino nitrogen. 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, 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 glutamate into all the amino acids. The results of Lewis and Patte 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. The $^{15}$N studies of Baker and Thompson 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, phosphoenolpyruvate or oxaloacetic) in the presence of a reductant or to glutamate to form glutamine and pass into the chain. The strong light dependence of glutamate synthase 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.

We thank Mrs Shirley Burgess for technical assistance and Dr D. O. Hall of Kings College, London for a gift of ferredoxin. P. J. L. was supported by a Pickering Research Fellowship.

*Note added in proof: Since this paper was submitted we have become aware of a report 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 and that senescence is under evolutionary control. Orgel 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 number...
number of divisions. The life of a culture of cells from a tissue explant can be divided into three phases. 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. Not only is the growth rate of non-dividing cells higher in phase III cells than phase II cells, but the growth rate of dividing cells is reduced; so all cells of the culture are affected by senescence. Phase II cells, however, are unaffected by being mixed with phase III cells.

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. 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.

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. 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. Serial transplants of normal tissue into young inbred animals die out and the death of the tissue is related to number of cell divisions rather than to its metabolic age. 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.

Hayflick postulated that organisms age because their cells age 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, while cells of normal people of similar age double 35 times. Cell senescence is not necessarily the only cause of organisic 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, 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. Benedict and Benditt 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 three individuals with heterozygous for two electrophoretic variants of the X-linked glucose-6-phosphate dehydrogenase. There is random inactivation of one of the X chromosomes at about 10,000 cell stage of embryo development, consequently each heterozygous female has a mixture of cells with one or the other X chromosome inactivated. When small pieces (0.1 mm³) were taken from the aorta wall, both enzyme types were present. But when larger pieces from plaques (0.5 mm³ to 20-30 mm³) were taken, most of them had only one isoyme present. It is unlikely that these plaques are scars formed where the artery had been injured, since hypertrophic scar tissue is polyclonal.

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 and would start dividing. As the single cells 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.

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 their 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. Many leiomyomas seem to originate near arteries and thus may have arisen from cells from arterial walls which have escaped into the myometrium 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', must be able to explain why cultures of transformed cells are not severely enough affected by the errors to senesce and why normal cells cannot 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 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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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. 1a). In 1% serum, myotubes formed after 2 d and in 10% serum they appeared after 4 d (Fig. 1c 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. 1b, 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.

Insulin stimulates myogenesis in a rat myoblast line

The clonal rat myoblast cell line L₄ isolated by Yaffe 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. When L₄ myoblasts fuse the specific activity of adenylate cyclase decreases and cyclic AMP dibutyryl cyclic AMP and theophylline reversibly inhibit the differentiation process. In primary cultures of rat muscle cells, a decrease in cyclic AMP has been reported at the time of fusion. 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 and the effects of the hormone on muscle metabolism, we have examined the influence of insulin on myogenesis of cultured L₄ myoblasts. The hormone replaces partially the serum requirement for morphological differentiation of primary cultures of chick embryo myoblasts, and now we have found that it stimulates fusion and the specific activity of creatine kinase in L₄ myoblasts in the presence or absence of serum.

Fig. 1 Effect of insulin and serum on myoblast fusion. Rat myoblast L₄ cells (from Dr. S. Heinemann, Salk Institute) were grown at 37°C in a humidified incubator under a 5% CO₂ 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 × 10⁶ cell cm⁻² in 35 mm dishes. This medium was removed 24 h later, the dishes were rinsed with phosphate-buffered saline, and reincubated under the following conditions, a and b. Serum-free medium containing 1.5 μg 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.

Les Euphausiacés du Pacifique équatorial et sud tropical
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Aspects du contact forêt-savane dans le centre et l’ouest de la Côte d’Ivoire. Étude descriptive
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with nuclei located more centrally. They also tended to degenerate more rapidly.

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. 2b). The increase in creatine kinase activity (as well as in morphological fusion) was delayed by about 24 h in cultures containing 10% serum (Fig. 2c). 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. 2b and c). In other experiments the glycogen phosphorylase content of myotubes, detected histochemically, 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. Since under similar conditions with the L4 myoblast line we observed a marked stimulation of creatine kinase activity by insulin, either there are differences in the response of insulin to primary cultures and L4 cultures, 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. 3a) 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. 2c). 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 3b shows that insulin had to be present for more than 24 h to stimulate significantly the creatine kinase specific activity, and longer exposure resulted in greater enzyme activity.

Insulin stimulates many cellular activities, especially in muscle cells, and its observed effect on myogenesis in both L4 cells and primary cultures 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. In the case of L4 myoblasts, however, cell density and protein synthesis are higher in 10% serum 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 and degradation and cyclic GMP levels in different cell types. Therefore it is attractive to suppose that such effects account for the stimulation of myogenesis in L4 myoblasts reported here, and perhaps for the insulin-promoted differentiation of mammary gland in organ.
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)\(^3\). The improvement in human erythrocyte survival in these animals is due to EP-induced impairment of reticuloendothelial function\(^4\) and to suppression of intravascular hemolysis through CVF inhibition of the third component of complement\(^5\). The half-life 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\(^6\).

Application of this animal system to the study of human sickle cell anemia erythrocytes (sickle cells) has verified many of their known survival characteristics in man\(^7\). These include a short intravascular survival, an improved survival with in vivo hyperoxemia 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)\(^10,11\). In contrast, the majority of sickle cells seems to be relatively insensitive to 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 51Cr survival curve of the transfused sickle cells assumes a characteristic 'saw tooth' pattern with peaks during hyperoxia and valleys during hypoxia\(^12\).

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

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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 51Cr-labelled erythrocytes (RBCs) from one of three patients with sickle cell anemia. 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 51Cr human sickle cells—were removed by cardiac puncture. The RBCs were washed with physiological saline, adjusted to a 50% suspension in saline, and then 3 ml were retransfused into recipient animals. Following this, all rats were exposed to a minimum of three hypoxia–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 51Cr RBC survival expressed as % activity found in the rats' blood immediately before exposure to the hypoxia–hypoxia cycles. The abscissa measures time (h) after start of the first hypoxia–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 behavior initially. After the third cycle, an O\(_2\)-sensitive population developed in the transfused sickle cells. c. The survival in the rats' blood of 51Cr-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 reticulocytes obtained from hypoxic donor animals do not exhibit the saw tooth pattern during the initial alternating periods of hypoxia 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% O2 and transfused into recipient animals (not shown in the figure) retain an O2 sensitive population. Continuation of the hypoxia–hypoxia cycles in the recipient rats with O2 insensitive transfused 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 O2-sensitive sickle cells to total sickle cells is similar to that in the original population. Wide changes in O2 tension seem to be necessary for the appearance of an O2-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 O2-insensitive transfused sickle cells in rats maintained on room air will remain resistant to O2 changes until they have been subjected to several hypoxia–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 O2-sensitive sickle cells from the animals' circulation during exposure to low O2 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-thalassemia and with sickle-Hb C disease. In contrast, none of eight individuals with sickle trait studied thus far has shown this type of response.

The observations reported here demonstrate that a proportion of O2-insensitive sickle cells can be transformed into an oxygen sensitive population when challenged with repeated hypoxia–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 anemia may be a factor of the relative number of sickle cells that are oxygen sensitive with regard to their capacity to sequester. 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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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 dy²/dy³ quadriiceps. 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 dy²/dy³ triceps culture. All photographs were taken at 7 d in vitro, under identical conditions.

regeneration, Hamburch et al." came to the conclusion that muscle from dystrophic (dy²/dy³) mice exhibited a capacity to regenerate similar to normal muscle, Gallup et al.°, 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, using mouse chimeras (SWV+C57Bl/6J dy²/dy³), 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.° and Hamburch et al." I cannot, however, agree with any of the findings or conclusions of Gallup et al.°. Finally, I am in agreement with Peterson that dy²/dy³ 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 (dy²/dy³) 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.°

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° and neural control of the histochemical properties of fully differentiated extrafusal muscle has been shown to be considerable. 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 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,° 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.
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 multinucleate 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.

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 x 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 transversely 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 chick 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 stopper, 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 PFAH.

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 initiated set up, the remainder have failed to grow. In all cases parallel extrafusal cultures were also obtained.

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 not at 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. 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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Figure 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 PGE₂ 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 μg ml⁻¹ streptomycin in Nunc Petri dishes. Transport was measured on cells attached to 30 mm dishes. Confluence 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 (thatched 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 ³¹P-phosphate (75 μCi ml⁻¹ 3.75 μCi ml⁻¹) and ¹⁴C-uridine (29 nCi ml⁻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. 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⁺) ATPase, 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 E₁ (PGE₁), 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, PGE₁ 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 results16-18 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 cells19. 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 cells19-21 and increases cyclic GMP in different tissues22 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 pleiotropic role for cyclic AMP in the control of all the changes in membrane transport produced by serum. In fact from the present and previous findings23-25 it is possible to define at least three different mechanisms of control, that can be differentated 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 these 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.

L. J. de A. is a Fellow of the Leukemia Society of America, Inc. We thank Drs R. Burk, R. Dubecco and M. Stoker for critical discussions and Miss H. Wiltshire for technical assistance. PGE₁ and SQ 20006, a new inhibitor of cyclic 3',5'-nucleotide phosphodiesterase activity were gifts of Dr Pike, Upjohn, and Dr Chasin, Squibb, respectively.

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

<table>
<thead>
<tr>
<th>Additions</th>
<th>Serum</th>
<th>SQ 20006</th>
<th>Uptake rate (c.p.m. per dish)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-</td>
<td>-</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>890</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>520</td>
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<td>-</td>
<td>-</td>
<td>280</td>
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<tr>
<td></td>
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<td>-</td>
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<tr>
<td></td>
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<td>+</td>
<td>7002</td>
</tr>
</tbody>
</table>

* 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.
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. It therefore affects 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. We have found that division in the fission yeast, Schizosaccharomyces pombe, is associated with a doubling in magnesium concentration within the cells. 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 Analal acetone and adding 1 ml of absolute ethanol. 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, 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. 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 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.

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. The homogenate was centrifuged at 40,000 gR (x), 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 much lesser extent, magnesium, 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 necessary to promote the breakdown of microtubules involved in nuclear
division and cell plate formation\(^1\) and the 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\(^1,2\). Here we present data on the membrane antigens of mouse liver cells solubile 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.

Livers 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\(^4\) (Fig. 1a). Before analysis all the TFs were treated 10–20 times and anti-gastro sera were treated four times. Each TF of mouse liver was checked by the precipitin reaction with 40 samples of rabbit anti-gastro 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-gastro 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 immunofluorography 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 immunofluorography. It is not detected in concentrated saline extracts of mouse liver, kidney, spleen or intestine by either the agar precipitation test or immunofluorography.

BCCA was partly purified by agar-gel preparative electrophoresis\(^8\). The combined and concentrated TFs of the mouse liver were electrophoresed in buffered 1% agar (0.5 N barbital buffer, pH 8.6). The electrophoretic fractions (in the region of β-globulin) with maximal BCCA concentration and minimal amounts of other antigens were collected and combined.

This partially purified BCCA and the anti-gastro serum containing antibodies predominantly to BCCA were used for the preparation of monospecific antibodies to BCCA\(^9\).

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. 1b). The Kupffer 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-gastro 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. 1c).

In the sections of intestine (Fig. 1d) 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](image-url)
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. 1e).

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. They also found it in kidney proximal tubule cells. Another antigen of rat bile capillaries has been reported. 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 cells1-3 and cell-free systems4-6. Ricin inhibits peptide chain elongation by catalytically inactivating the 60S subunits of ribosomes2-7. It is a galactose-binding glycoprotein of molecular weight 60,000, containing two nonidentical subunits linked by disulphide bridges8-11 and seems to be identical to the lectin R. communis agglutinin II (RCA11) isolated by affinity chromatography8-11. Ricin, or RCA11, has been used to suppress the growth of ascites tumour cells in vivo and, as a cell surface probe for oligosaccharide receptors12-15 containing terminal D-galactose or N-acetyl-D-galactosamine-like saccharides14-16,17, I have found that RCA11 enters mammalian cells by endocytosis (similar to the findings of Oliver et al.) and is then released into the cytoplasm, where it apparently acts directly on protein synthesis.

Although 125I-labelled RCA11 binds rapidly to mammalian cell surfaces18,19 (saturation <10 min at 5°C)20, 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 RCA11 toxicity21,22. Also, the toxic action of RCA11 can be prevented by addition of lactose23 or antitoxin24 within 15-30 min. In cell-free systems RCA11 suppresses protein synthesis almost immediately (~1.5 min)24,25, and this action is not sensitive to RCA11 saccharide inhibitors25. To investigate the ultrastructural events subsequent to cell binding I have used an electron-dense ferritin-conjugate of RCA11 (FeRC11)26,27.

FeRC11 was synthesised as described before26,27 and purified by affinity chromatography on Biogel A-1.5 m28. After elution of column-bound FeRC11 by 0.2 M D-galactose, the conjugate was dialysed extensively. Affinity purified FeRC11 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 FeRC11 conjugate was not as active in suppressing protein synthesis as native RCA11; this might reflect some inactivation during the coupling process or in the presence of ferritin with the enzymatic properties of RCA11. But, as with RCA11, the conjugate was blocked by the addition of D-galactose to the incubation medium (Table 1). To study the ultrastructural interaction of FeRC11 with cells, the conjugate was added to 0.02% EDTA-dissociated, washed, confluent grown murine 3T3 cells suspended in Dulbecco's modified Eagle's minimal essential medium29 (DMEM) containing 5% calf serum for various times at 4°C, 20°C or 37°C. Alternatively, cells were pulsed with FeRC11 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 except 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 FeRC11 labelling. Incubations were terminated by washing the cells in fresh DMEM and then adding 1.5% glutaraldehyde in sodium phosphate-buffered saline (pH 7.2, 310 mosmol). Fixation proceeded for at least 1 h, the last 30 min at the incubation temperature. The cells were washed again and postfixed in 1% osmium tetroxide in 0.1 M sodium phosphate buffer, pH 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 FeRC11 for 5-30 min at 0-4°C or 4°C (5-10 min at 20°C-37°C) before fixation showed continuous labelling of RCA11 receptors, exclusively at the cell surface (Fig. 1d). Labelling was specific as D-galactose inhibits conjugate binding (Fig. 1b), as previously demonstrated30. Labelling at higher temperatures (20°C-37°C) resulted in eventual lectin-induced receptor clustering of some but not all RCA11 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. 1e). At times greater than 60 min at 37°C most FeRC11 molecules were localised inside intact or broken endocytotic vesicles and free in the cell cytoplasm (Fig. 1d).

To determine the sequence of FeRC11 receptor interaction and endocytosis, pulse labelling experiments were performed. Cells were labelled with FeRC11 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 FeRC11 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 FeRC11 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 FeRC11-labelled cells to 37°C following labelling and then washing at 0-4°C, progressively less FeRC11 could be removed from cells with time by addition of D-galactose. Also, the location of FeRC11 changed from exclusively extracellular in a dispersed distribution (0-5 min after shifting to 37°C), to both extracellular and intracellular (clustered at the

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

<table>
<thead>
<tr>
<th>Pretreatment*</th>
<th>Time (min)</th>
<th>Incorporation of 3H-Leu into protein c.p.m.†</th>
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<tr>
<td>None</td>
<td>0</td>
<td>111,700</td>
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<td>†RCA1</td>
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<td>105,100</td>
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<td>103,300</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>5,100</td>
</tr>
<tr>
<td>†Fer-RCA1 + ϑ-galactose</td>
<td>60</td>
<td>85,700</td>
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*Confluently grown 3T3 cells were removed from substrate with 0.025% 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-RCA1 (≈100 μg ml⁻¹ total protein) for various times in DMEM at 37°C in a CO₂ incubator.

†Cells were treated with Fer-RCA1 and washed in DMEM minus leucine and incubated in DMEM plus 3H-leucine (3 μCi ml⁻¹) for 60 min at 37°C in a CO₂ incubator. The cells were washed three times in TC-PBS and dissolved in 1 ml of 1 M 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 GF/C glass filters with vacuum. The filters were washed twice with 10% 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 μg ml⁻¹
††††Final concentration (total protein), approximately 100 μg ml⁻¹

Fer-RCA1 that had moved into cells by endocytosis was not susceptible to removal by addition of ϑ-galactose.

Results consistent with these have been obtained with 125I-RCA1 (ref. 21). After a 10 min pulse (1 μg ml⁻¹ for I-RCA1) at 4°C, approximately 95% of the cell-bound 125I-RCA1 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 RCA1 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-RCA1 (Table 1).

In cell-free protein synthesis systems inhibition was almost complete within 1-2 min under these conditions. 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.

Refines et al. proposed a two-step model for the toxic action of abrin 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 and endocytosis are important in the entry of toxin into mammalian cells. The ultrastructural fate of Fer-RCA1 after endocytosis suggests that the lectin molecules are released from endocytic vesicles, perhaps by breakdown. Lysosomes do not appear to be involved in this process, because I did not observe fusion of lysosomes with the endocytic vesicles; however, this cannot be ruled out for all vesicles. The fact that simple RCA1 binding to cells is not sufficient to cause inhibition of protein synthesis suggests that the endocytosis and release into the cell cytoplasm of RCA1 where it can directly interact with polysomes is the natural mode of action of this toxin. Other toxins such as diphtheria toxin may enter the cell to act in similar fashion.

This work was supported by a grant from the Human Cell Biology Programme of the US National Science Foundation, a contract from the Tumor Immunology Program of the

Fig. 1 Fer-RCA1 binding to murine 3T3 cells at 37°C. a, After a 10 min incubation, ferritin was localized exclusively at the cell surface more or less randomly distributed. Note that the vesicle to the left is unlabelled. b, Labelling is specific to ϑ-galactose abolished Fer-RCA1 binding activity. c, Between 30 and 60 min of incubation Fer-RCA1 clustered at the cell surface and endocytosis has begun. d, After 60-90 min incubation ferritin was localized inside the cell in intact or broken endocytic 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
Serum dopamine β-hydroxylase activity in developing hypertensive rats

Spontaneously hypertensive (SH) rats have increased tyrosine hydroxylase and dopamine β-hydroxylase (DBH) activities in the adrenal glands. They also show a pronounced hypertensive reaction to the specific microbical inhibitors of these enzymes. The results suggest some involvement of the sympathetic 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, does not differ significantly from that of normotensive Wistar rats.

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 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. 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 10,000g for 10 min. The enzyme activity was assayed by sensitive dual-wavelength spectrophotometry using tyramine as substrate. This method permits the assay of the maximum velocity at a saturated substrate concentration (20 mM) under the optimum pH (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 pH 5.0, 100 μl of 1 M N-ethylmaleimide, 50 μl of 20 μM CuSO4, 50 μl of 0.2 M sodium fumarate, 50 μl of 0.2 M ascorbic acid, 50 μl of 20 mM paraglycin, 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 x 10 cm) of Dowex-50-H⁺ (packed volume, 0.2 ml), and converted by NaIO4 (10 μl 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. 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.

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. 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

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>DBH activity (nmol min⁻¹ ml⁻¹ serum ± s.e.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wistar-Kyoto rats</td>
</tr>
<tr>
<td>3</td>
<td>SH rats</td>
</tr>
<tr>
<td>6</td>
<td>1.18 ± 0.03</td>
</tr>
<tr>
<td>12</td>
<td>0.44 ± 0.02</td>
</tr>
<tr>
<td>14</td>
<td>0.19 ± 0.04</td>
</tr>
<tr>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>53</td>
<td>0.20 ± 0.02</td>
</tr>
</tbody>
</table>

*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 profoundly. 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.

We thank Professor K. Okamoto and Dr. Y. Yamori (Kyoto University, Kyoto) for spontaneously hypertensive rats and Wistar-Kyoto rats and Miss N. Yamada and Miss M. Suzuki for technical assistance. This work was supported by a grant from Science and Technology Agency, Japan.

**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. Experiments 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. It was therefore surprising when, in two laboratories (St Andrews and Kjell), we independently found that the two homologous giant metacerebral neurones, which contain serotonin and are thought to use it as a neurotransmitter, 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.

Experiments at Kjell, ChAc was assayed as described by Fonnun with modifications in the incubation medium to yield optimal activity for Helix. In experiments at St Andrews, ChAc was assayed by the same method as modified by Coggeshall et al. using the following incubation medium (final concentration): 200 μM [3H]-acetyl-CoA, 20 mM choline chloride, 300 mM NaCl, 1 mM EDTA, 2H₂O, 80 mM sodium phosphate buffer (pH 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 [3H]-acetyl-CoA, 0.2 mM dL-1-α-C-DOPA, 8 mM choline bromide, 250 mM NaCl, 20 mM sodium phosphate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Activity (pmol cell⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.3 ± 6.3 (3)</td>
</tr>
<tr>
<td>Incubation medium + AChE-</td>
<td>1.2 ± 0.3 (3)</td>
</tr>
<tr>
<td>eserine</td>
<td></td>
</tr>
<tr>
<td>Incubation medium—choline</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Incubation medium +</td>
<td></td>
</tr>
<tr>
<td>Acetylcholine (1.25 mM)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Incubation medium +</td>
<td>2.5 (3)</td>
</tr>
<tr>
<td>Chlortoacetycholine (0.125 mM)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Incubation medium +</td>
<td>2 (3)</td>
</tr>
<tr>
<td>CoA (100 mM)</td>
<td>10.8 ± 3.7 (3)</td>
</tr>
<tr>
<td>Incubation medium +</td>
<td>2.2 (3)</td>
</tr>
<tr>
<td>Incubation medium +</td>
<td>3.3 ± 2.9 (3)</td>
</tr>
<tr>
<td>ACh (20 mM)</td>
<td>13.0 ± 2.9 (3)</td>
</tr>
<tr>
<td>Incubation medium +</td>
<td>2.5 (3)</td>
</tr>
<tr>
<td>MgCl₂ (100 mM)</td>
<td>8.9 ± 2.4 (3)</td>
</tr>
<tr>
<td>Incubation medium +</td>
<td>2.5 (3)</td>
</tr>
<tr>
<td>CaCl₂ (100 mM)</td>
<td>2.5 ± 2.3 (3)</td>
</tr>
<tr>
<td>Heat denaturation (1 h at 90°C)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>TCA precipitation</td>
<td>2 (3)</td>
</tr>
</tbody>
</table>

Results are expressed as means ± s.e.m. The numbers in parentheses refer to no. of values used for mean.
buffer (pH 7.4), 0.1 mM eserine, 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 \(\text{K}^{+}\) as before. Animals were maintained in artificial hibernation by storage in a humid environment at 10\(^{\circ}\) C. The dissection was performed as described elsewhere. In some experiments, the removal of single cells was expedited by conducting the dissection under 70% ethylene glycol (\(-20^{\circ}\) C). Enzyme activities were found to be unaffected by the choice of dissection solution, as noted earlier. 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; second, no activity was found in the absence of choline; third, no activity was found in the presence of acetylcholinesterase and the absence of choline substrate inhibitor and, fourth, \(\text{K}^{+}\) extraction completely separated ACh from acetylputaminine and other possible acetylated contaminants.

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, acetylcholinesterase, and chloroacetylcholine, in concentrations known to similarly affect the partially purified ChACh from whole snail brain. The latter two substances should be regarded as fairly specific ChACh 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 posterior-dorsal surface of the visceral ganglion (known as cell no. 21 (ref. 1) on cell no. 4 (ref. 2)), but comparable to that of the medial buccal cell (cell no. 4 (ref. 1)), which was considered to have a relatively low ChACh activity. Other cells known to contain large amounts of serotonin or AAD, such as the one localised near the right parietal ganglion in the posterior-dorsal surface of the visceral ganglion (known as cell no. 11 (ref. 1) on cell no. 4 (ref. 3)), contained insignificant ChACh 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 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.P., unpublished).

---

**Table 2** Enzyme activities of single neurones using both single and double assays for ChACh and AAD in *Helix aspersa*

<table>
<thead>
<tr>
<th>Neurone</th>
<th>Location</th>
<th>ChACh activity pmol cell(^{-1}) h(^{-1})</th>
<th>Double assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSC</td>
<td>Ventral surface of metacerebral ganglion, near input of inner lip nerve</td>
<td>22.3 ± 3.4* (4)</td>
<td>ChACh activity pmol cell(^{-1}) h(^{-1})</td>
</tr>
<tr>
<td>Medial buccal cell</td>
<td>Lateral edge of dorsal surface of buccal ganglia.</td>
<td>21.0 ± 5.0 (4)</td>
<td>AAD activity</td>
</tr>
<tr>
<td>Cell 21</td>
<td>Medial of group of three giant neurones Posterior-dorsal surface of visceral ganglion, between inputs of cutaneous pallial nerve and visceral nerve</td>
<td>65.6 ± 7.9 (5)</td>
<td></td>
</tr>
<tr>
<td>Cell 11</td>
<td>Posterior-dorsal surface of visceral ganglion, bordering right parietal ganglion</td>
<td>&lt; 2 (5)</td>
<td></td>
</tr>
<tr>
<td>Posterior buccal cell</td>
<td>As medial buccal cell. Posterior of group of three giant neurones Control extract from area of GSC dissection (0.5 µl)</td>
<td>&lt; 2 (5)</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses refer to number of determinations used to derive figure.
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, 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. 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 comprised 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)1, WA-1a-D3b (A9 × WI-38), human embryonic lung fibroblasts) and IL-11-3b (mouse L cell line LM(K)2 × IMR 32, human neuroblastoma).

For regional localisation studies we used two hybrid lines from the WL series16, 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(K)2 with WI-38. They were maintained in HAT selective medium18 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 assigned19,20. 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              | PepC            |
| 2              | IDH-1           |
| 3              | MDH-1           |
| 4              | ME-1            |
| 5              | MPI             |
| 6              | GOT             |
| 7              | LDH A           |
| 8              | Pep B           |
| 9              | NP              |
| 10             | APRT            |
| 11             | ADP              |
| 12             | GPI             |
| 13             | ADA             |
| 14             | SOD-1           |
| 15             | G6PD            |
| 16             | PGK             |

Declassified and Approved For Release 2014/01/09 : CIA-RDP79-00999A000200010001-4
Table 2
Number of hybrids showing presence (+) or absence (−) of the two given human enzymatic activities

<table>
<thead>
<tr>
<th></th>
<th>+/+</th>
<th>+/−</th>
<th>−/+</th>
<th>−/−</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>GaK/IDH-1</td>
<td>5</td>
<td>18</td>
<td>3</td>
<td>24</td>
<td>50</td>
</tr>
<tr>
<td>GaK/MDH-1</td>
<td>6</td>
<td>17</td>
<td>7</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>GaK/Pep A</td>
<td>17</td>
<td>6</td>
<td>10</td>
<td>17</td>
<td>50</td>
</tr>
<tr>
<td>GaK/TK</td>
<td>16</td>
<td>2</td>
<td>0</td>
<td>20</td>
<td>38</td>
</tr>
</tbody>
</table>

Hybrids assayed for IDH, MDH-I 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. 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 solv 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. Thymidine kinase activity was assayed on Cellogel (Chemotron, Milan, Italy) using the method of Migeon et al. Electrophoresis was carried out at 4°C for 4 hours at 30 V cm⁻¹ using a buffer of 50mM TES (N-tris(hydroxymethyl)methyl-2-aminoethane sulphonic acid) adjusted to pH 6.2 with triethanolamine. Our method used to assay galactokinase activity on vertical starch gels has been described. Enzymatic analysis of the hybrids from series 41, 50c, AIM and YA indicated that galactokinase (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 GalK 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 21q-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.

Table 3
Human GaK activity and human chromosomes present in hybrid cell lines

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Human GaK</th>
<th>Human chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>JFA-16a-5</td>
<td>+</td>
<td>10 11 2 3</td>
</tr>
<tr>
<td>JFA-16a-8</td>
<td>+</td>
<td>11 12 3 4</td>
</tr>
<tr>
<td>JFA-16a-15b</td>
<td>+</td>
<td>3 4 5 6</td>
</tr>
<tr>
<td>JFA-16a-19</td>
<td>+</td>
<td>5 6 7 8</td>
</tr>
<tr>
<td>JFA-16a-21</td>
<td>+</td>
<td>7 8 9 10</td>
</tr>
<tr>
<td>JFA-14a-5</td>
<td>+</td>
<td>9 10 11 12</td>
</tr>
<tr>
<td>JFA-14a-10c</td>
<td>+</td>
<td>11 12 X 13</td>
</tr>
<tr>
<td>JFA-14a-12</td>
<td>+</td>
<td>13 14 15 16</td>
</tr>
<tr>
<td>JFA-14a-13</td>
<td>+</td>
<td>15 16 17 18</td>
</tr>
<tr>
<td>JFA-14a-23b</td>
<td>+</td>
<td>17 18 19 20</td>
</tr>
<tr>
<td>JFA-14a-6b</td>
<td>+</td>
<td>19 20 21 22</td>
</tr>
<tr>
<td>WA-1a-D6</td>
<td>+</td>
<td>20 21 22 Y</td>
</tr>
<tr>
<td>IL-1-5</td>
<td>+</td>
<td>21 22 Y</td>
</tr>
</tbody>
</table>

Karyotypes of hybrids were analysed using air dried preparations stained with quinacrine mustard or Atebrin to produce fluorescent banding of the chromosomes and stained subsequently with Giemsa to reveal constitutive heterochromatin. 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.
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* and are closely linked also in yeast, *Saccharomyces cerevisiae*, it is interesting to know whether the corresponding genes are linked in man. Human galactose-1-phosphate uridylyltransferase (EC 2.7.7.12) has been assigned to 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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Fig. 1 Galactokinase assay of five WL-24a-2-A subclones. After electrophoresis in citrate phosphate buffer at pH 6.8, the gel was incubated in the reaction mixture of Mayes and Guthrie. C-galactose-1-phosphate produced by the enzyme was then precipitated in the gel with lanthanum chloride and subsequently detected by autoradiography. 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.

Fig. 2 Chromosome 17 as given by the Paris Conference (1971) (ref. 29) indicating the assigned map position for GaK.
5-Methylcytosine localised in mammalian constitutive heterochromatin

5-Methylcytosine (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, no more than 1.5% of the bases in the mouse and even less in the human. 5-MeC itself is not incorporated directly into DNA. Instead, specific deoxyctydylc 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.

In the mouse, as in other mammals, virtually all of the 5-MeC is in the 5-MeCpG doublet, which is distributed non-randomly in the DNA. Mouse satellite DNA contains about 3% 5-MeC in comparison to about 1.5% in main band DNA and is located at the centromeric end of nearly every chromosome.

Antibodies to 5-methylcytosine (anti-5-MeC) are available and can react with 5-MeC residues in single-stranded DNA. Earlier we showed that antibodies specific for particular purine or pyrimidine bases, which react with denatured DNA but not native DNA, can bind to fixed metaphase chromosomes. The major sites of binding can be localised by immunofluorescence. 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.

We have now investigated the chromosomal location of 5-MeC residues in both the mouse and human. Chromosome preparations which had been either ultraviolet irradiated or heated to 85°C in 0.07 M Sorenson’s buffer at pH 6.5 for 15 or 30 min and chilled suddenly to prevent reassocation, 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. 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, 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.

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 Wettmur and Sutton and McCallum. Sutton’s estimate of 8–13 nucleotides 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 classes 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 NaHPO4, 15 ml 0.25 M KH2PO4) in 2,400 ml distilled water, pH 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 cardiac condenser, BG-12 exciter filter and K530 barrier filter. The photographs were taken on Kodak Plus X film with a 2 min exposure, developed with Acufine and printed on Ilford No. 4 grade paper.
of human satellite II DNA as revealed by in situ DNA–RNA hybridisation[1], whereas the corresponding 5-MeC-positive region of chromosome 9 contains both satellite II DNA[2] and satellite III DNA[3]. 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 repetitive, 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]. The very small size of the chromosomal DNA in yeast[4,5] and the availability of temperature-sensitive DNA synthesis mutants[6] 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: cdc4, cdc7 and cdc8 (refs 8,9). If synchronous cultures of cells carrying mutations in cdc4 or cdc7 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]. It has been suggested, therefore, that the gene functions cdc4 and cdc7 are required for the initiation of nuclear DNA synthesis[10]. The gene function cdc8 is necessary for DNA propagation since cells carrying this mutation abruptly shut off nuclear DNA synthesis when shifted to the restrictive temperature[11].

When DNA molecules from cdc4 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 cdc7 at the restrictive temperature (35°C), 144/150 molecules were simple linear structures. Six of the molecules contained replication forks, possibly the result of some leakiness of DNA syn-
initiation mutants cdc4 and cdc7 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 indicate that cdc4 and cdc7 may be true DNA initiation mutants.

The findings with cdc8, the DNA propagation mutant, were quite different. Approximately half (114/240) of the DNA molecules isolated from cdc8 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. Figure 3 is a histogram of center-to-center distances between bubbles in molecules which contain more than one bubble.

The high proportion of DNA molecules with replication bubbles found in experiments with cdc8 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 suggesting that DNA synthesis may be initiating in cdc8 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 cdc8 at the restrictive temperature is less than 1% of the normal rate (C.S.N., unpublished observations). An alternative explanation for the small replication bubbles, is that the cdc8 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 cdc8 at the restrictive temperature which contain small replication bubbles confirms the classification of cdc8 as a DNA propagation mutant. Hartwell has recently classified another cell cycle mutant, cdc21, as a possible DNA propagation mutant on the basis of incorporation kinetics. Preliminary

Fig. 1. Small DNA replication bubble in DNA isolated from a temperature-sensitive DNA propagation mutant (cdc8) at the restrictive temperature. A culture of cdc8 (strain 195-1) was grown at 23°C in 10 ml of Y-minimal media 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 x 10^9 cells ml^-1, the culture was shifted to 38°C for 3 h. Cells were then collected and spheroplasted as described previously. 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. Methods for spreading DNA for electron microscopy have been described. Each branch of the bubble in the photographed molecule is 0.4 μm in length; arrows show the forks. Nuclear DNA was prepared from cdc4 (strain 135.1.1) and cdc7 (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. For cdc8, 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 2-5% of the DNA molecules in an asynchronous culture are replicating at any given time, most of the DNA molecules observed in the cdc8 experiment should contain no replication structures before the shift to the restrictive temperature.

thesis at the restrictive temperature. This allele of cdc7 (4008) is somewhat leaky at 36°C (ref. 9) synthesizing nuclear DNA at approximately 10% of the normal rate (C.S.N., unpublished observations). When the experiment is done at 38°C with cdc7, 149/150 DNA molecules were simple linear structures.

Hartwell 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

Fig. 2. Histogram of bubble sizes from DNA molecules isolated from cdc8 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 in DNA molecules containing more than one bubble. Measurements were made on DNA molecules isolated from cdc8 at the restrictive temperature.

experiments with cdc1 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 cdc8 but not in the initiation mutants cdc4 and cdc7 cannot be explained by suggesting that cdc8 is more leaky at the restrictive temperature than cdc4 or cdc7. As already discussed, at 36°C, cdc7 is considerably more leaky than cdc8. These results, therefore, also imply a qualitative difference in the leakiness of cdc7 and cdc8 (ref. 10). In cdc8 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 cdc7 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 promoter in the order of 16S, 23S, SS (for review, see ref. 1). In this report we determine the positions of the 16S (0.55 × 10⁶ dalton), 23S (1.10 × 10⁶ dalton), and SS rRNA (0.40 × 10⁶ dalton) cistrons on their unit map (scritton) by the ultraviolet light mapping technique 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 promoter. Therefore, the expression of promoter distal genes is more sensitive to ultraviolet irradiation than that of promoter proximal ones.

Rates of synthesis of 23S, 16S and SS rRNA after irradiation of E. coli with various doses of ultraviolet light were determined by 3H-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 3H-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 or SS rRNA. Synthesis of SS rRNA was very sensitive to ultraviolet light. A greater reduction in 23S RNA synthesis compared with 16S RNA synthesis after ultraviolet irradiation has been observed previously.15–14

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 100 and 170% at the ordinate, yield inactivation coefficients of -3.7 × 10⁻³ s⁻¹, -0.12 s⁻¹, and -0.12 s⁻¹ for synthesis of 16S, 23S and SS 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 SS RNA 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 are in a single E. coli chromosome and are structurally identical.

We suggest that the shoulders of the survival curves are caused by the following effect. Not all ribosomal RNA scrittons are transcribed at the maximal rate in the unirradiated cell. As the number of unhitted scrittons decreases within a cell with increasing ultraviolet dose, dormant rRNA scrittons become activated, or the remaining rRNA scrittons are utilised better. When cells are irradiated for more than 10 s the rates of rRNA synthesis are exclusively determined by the number of unhitted cistrons for the mature rRNA species.

16S, 23S and SS RNA 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 B4.1 (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 x 10^6 cells per ml, ^14C-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 (5,000 g, 15 min), resuspended in 30 ml fresh M-9 medium (approximately 10^6 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 G444413 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 cm^-2 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 mm^-1, Amersham-Searle) was added to a concentration of 1 μCi ml^-1 to each culture. At 12.5 min after the third uridine addition, the cultures were poured into ice cold centrifuge tubes, the cells were pelleted, resuspended in 2 ml 3mM EDTA (pH 7.2) and lysed by addition of 0.2 ml 10% Na dodecyl sulphate (SDS, Bio-Rad) in a 1 ml, 90°C incubation. RNA was extracted from the lysates with H_2O-saturated phenol, precipitated and resuspended in 0.3 ml 3mM EDTA. To these RNA preparations sucrose was added to 15% and SDS to 1%. Samples of 0.1 ml of each RNA preparation were layered onto 15 cm 2.6% acrylamide gels and electrophoresed at 70 V for 5.5 h; the gels were sliced into 2 mm fractions, digested in peroxide and counted in a liquid scintillation counter. The counts in the H-precursor-labelled RNA and ^14C-labelled RNA were corrected for interchannel spilllover. Right panel: tritiated uridine labelling of SS and 4S RNA as a function of ultraviolet irradiation dose. Samples of 0.1 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 SS and 4S RNAs. The major peak to the right of SS RNA is the 4S RNA 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 SS region were detectable at irradiation times in excess of 23 s (data not shown). O, ^1H c.p.m.; O, ^1C 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 x 0.55 x 10^6 daltons, 1.8 x 10^6 daltons away from the p30 promoter. Since the molecular weight of the 5S rRNA is 0.04 x 10^6 and since SS synthesis has essentially the same inactivation coefficient as 23S (Fig. 3), SS 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 promoter locus, we project a molecular weight of about 1.85 x 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 x 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. 3a.

with the 3' terminus of p30 at about 1.85 x 10^6 daltons. If the molecular weight of p30 were about 2.1 x 10^6 (ref. 17) the 16S rRNA could be preceded by approximately 0.9 x 10^6 daltons of non-conserved spacer RNA and the second spacer situated between 16S and 23S would be about 0.3 x 10^6 daltons (Fig. 3b). Alternatively the additional 0.25 x 10^6 daltons of non-conserved RNA could be located beyond the 3' end of the map as drawn (dashed line) in Fig. 3a. Figure 3a 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 promoter 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 SS rRNA can be calculated accurately, using the ^1C-prelabelled rRNA as an internal standard, from the expression:

\[
\text{% of control synthesis} = \frac{[\text{H} \text{c.p.m. RNA}_x]}{[\text{H} \text{c.p.m. RNA}_x]_{\text{control}}} \times 100
\]

where x refers to the species of rRNA under consideration, 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_1 = 3.7 x 10^{-3} s^{-1}, K_2 = K_3 = 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.
lesion per 1,000 DNA base pairs in the p30 cistron 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. Photoreactivation of ultraviolet-light-induced lesions which impair RNA synthesis in general has been reported earlier.

Actinomycin D has been used in studies of transcriptional linkage 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, 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 the experimental data. The transcriptional arrangement of the RNAs 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, 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 DNA1 and divide2. It has been estimated3,4 that chloroplasts contain many copies of DNA, but in higher plants, which contain large numbers of chloroplasts per cell5, it is not known when the DNA of chloroplasts replicates and how it is distributed when they divide. In the unicellular alga, Ochrotrichon, which contains a single chloroplast, 3H-thymidine is readily incorporated into chloroplast DNA and is distributed equally among the progeny chloroplasts. On the other hand, in Acraballaria, 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 remainder2.

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 light1. There is virtually no cell division during this time6.

Using 3H-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 extractions2.

The distribution of label amongst daughter chloroplasts was followed in freshly excised spinach disks (2 mm dia-
Table 1  
<table>
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<th></th>
<th>End of pulse</th>
<th>End of chase</th>
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<tr>
<td>Grains per chloroplast</td>
<td>23.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Labelled chloroplasts</td>
<td>100%</td>
<td>99%</td>
</tr>
<tr>
<td>Chloroplasts per cell</td>
<td>23</td>
<td>148</td>
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Spinach disks 2 mm in diameter were excised from the base of leaves 2 cm long and grown on sterile agar. 1 ml of 50 μCi 6-3H-thymidine (27 Ci ml⁻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⁻¹) 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 (pH 9) overnight at 60°C to separate the cells. The cells were spread on to slides, air dried and dipped in Ifford 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 3H-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 3H-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.

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.

Our results differ from those of Woodcock and Bogorad with Acetabularia where there are large variations in the DNA content of chloroplasts. Herrmann 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.

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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
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. A similar inhibition of the translation of exogenous viral or cellular mRNAs was reported for cell-free extracts from interferon-treated Ehrlich ascites cells. 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. 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 and to synthesis haemoglobin. 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. 1 Effect of interferon on Friend virus synthesis in unstimulated FSD1/F4 cells. Mouse interferon was prepared by the infection of L929 cells with Newcastle disease virus (NDV-California strain). Cell supernatants were dialysed against 0.1 M glycine–HCl buffer (pH 2.0) for 5 d and further purified by gel chromatography. The highly purified samples of mouse interferon (10 mouse reference research standard units per mg protein) were assayed by a plaque reduction test of vesicular stomatitis virus in mouse L929 cells. FSD1/F4 cells have been cultured as described previously 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. Total synthesis of C-type particles was analysed by measurement of 3H-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} 5,6-3H-uridine (Amersham; specific activity, 50 Ci mmol^{-1}) from 24–48 h. Cell supernatants were cleared from debris at 8,000g, 1 h 4°C. Pellets were resuspended in a buffer containing 0.1 M NaCl, 0.01 M Tris-Cl, 0.001 M EDTA, pH 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. O, 300 U ml^{-1} interferon; x, 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} (1), sucrose gradient. O, Untreated cells; x, mouse interferon 600 U ml^{-1}; Δ, 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.

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. 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. Erythroid differentiation of cells was estimated by staining cells with benzidine. a, Effect of interferon on virus synthesis in DMSO stimulated cells. O, DMSO 1.5%, no interferon; x, 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. O, DMSO 1.5%, no interferon; x, 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\(^4\). In some instances, however, interferon can be used to enhance neoplastic development\(^5\). Interferon also interferes with the transformation of fibroblasts by sarcoma viruses\(^6\). Mice with Friend erythroleukaemia have been reported to display a reduced capacity to respond to inducers of interferon\(^7,8\). No interferon could be detected in the supernatants of FSDF1/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 FSDF1/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. 1a, b). Heterologous chicken interferon does not reduce the virus yield (Fig. 1b). 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. 1c). The growth rate of the interferon treated cells was only slightly reduced.

When FSDF1/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). Interferon, however, depresses the virus synthesis below background level (Fig. 2a). 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. 2b).

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\(^4\) 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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7 Ostertag, W., et al., in Differentiation and Control of Malignancy of Tumor Cells (University of Tokyo Press, in the press).
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. This differentiation is demonstrated by morphological and biochemical alterations which are characteristic of normal erythroid maturation. 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% CO₂ and 95% room air. The cultures were passaged at 3 or 4 d intervals at a seeding concentration of 1 x 10⁶ cells ml⁻¹. DMSO (Fisher Sci.) was added (2%, v/v) 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 ⁵⁹Fe 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, and counted. This procedure provides an accurate assessment of the rate of haem synthesis by Friend leukaemia cells. We have already shown that during DMSO-induced differentiation of Friend erythroblastic leukaemia cells, increased iron incorporation into haem was coordinated with increase in the rate of synthesis of globin, haemoglobin, and globin mRNA. To assess the rates of DNA, RNA and protein synthesis, 5 µCi of ['H]thymidine, ['H]uridine or ['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 Shattakin. 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 erythroblastic leukaemia cells which had previously been grown in the presence of DMSO for 3 d (Table I). 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 ± 0.03 x 10⁶ cells ml⁻¹ on day 3 to 1.24 ± 0.04 x 10⁶ 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 ⁵⁹Fe; 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 and foetal liver erythroblasts. Although Ep had no effect on the rates of DNA and RNA precursor incorporation 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>
<thead>
<tr>
<th>Parameter</th>
<th>Mean % stimulation ± s.e.m.</th>
<th>No. experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Haem synthesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. CPM-⁵⁹Fe per plate</td>
<td>29 ± 4.6</td>
<td>29</td>
</tr>
<tr>
<td>b. CPM-⁵⁹Fe per 10⁶ cells</td>
<td>29 ± 4.1</td>
<td>29</td>
</tr>
<tr>
<td>(2) Cell number</td>
<td>+3 ± 2.4</td>
<td>29</td>
</tr>
<tr>
<td>(3) % Benzidine-positive cells</td>
<td>6 ± 4.7</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 1: Effect of Ep on erythroblastic leukaemia cells grown in DMSO medium

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, ⁵⁹Fe was added and 4 h later the haem extracted. For morphological studies, slides were made with a cytocentrifuge, were benzidine stained, 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:

\[(\text{Parameter Ep culture} - \text{Parameter no Ep culture}) \times 100\]

The % stimulation was determined for each individual experiment and the data so derived pooled and used to calculate the 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 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. 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. 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 \(^{12-13}\). 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

<table>
<thead>
<tr>
<th>Macromolecule</th>
<th>DMSO-containing cultures</th>
<th>Control cultures</th>
<th>Mean c.p.m.</th>
<th>Mean c.p.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per 10^6 cells ± s.e.</td>
<td>per 10^6 cells ± s.e.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) DNA</td>
<td>0</td>
<td>0</td>
<td>6,073 ± 1182</td>
<td>3,928 ± 138</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>7,474 ± 161</td>
<td>1,501 ± 34</td>
</tr>
<tr>
<td>(b) RNA</td>
<td>0</td>
<td>0</td>
<td>1,737 ± 147</td>
<td>5,018 ± 254</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>2,556 ± 174</td>
<td>5,381 ± 137</td>
</tr>
<tr>
<td>(c) Protein</td>
<td>0</td>
<td>0</td>
<td>4,408 ± 84</td>
<td>15,687 ± 310</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>6,653 ± 57</td>
<td>15,553 ± 428</td>
</tr>
<tr>
<td>(d) Haem</td>
<td>0</td>
<td>0</td>
<td>1,169 ± 128</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>1,796 ± 103</td>
<td>7</td>
</tr>
</tbody>
</table>

Suspension cultures were set up in bottles containing 40 ml each. After 3 days 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 erythroblastic 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* (Ehrenh.) Lev. (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 un contaminateduredospores. Vegetative growth and some sporulation were also obtained with an Australian isolate of wheat rust, race 126-ANZ 6, 7.

Williams et al. reported the first successful establishment of axenic colonies of the wheat stem rust fungus, race 126-ANZ 6, 7, starting with un contaminateduredospores 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*\(^{12-13}\) and *Melampsoraceae*\(^{14-15}\). 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. 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\(^{16}\). 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) in order to examine the factors controlling sporulation and to compare the amino acid requirements of the wheat and flax rust fungi.

Uncontaminateduredospores of both rusts were raised on susceptible hosts as described elsewhere\(^1\). 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 \(\mu\)m). No agar was employed. The \(pH\) of all media was adjusted to 5.0. Uredospores (1,000–2,000 \(mm^3\)) 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>
<thead>
<tr>
<th>Fungus</th>
<th>Medium</th>
<th>Aspartic acid*</th>
<th>Organic nitrogen source (mM)</th>
<th>Glutamine</th>
<th>Cysteine</th>
<th>Glutathione</th>
<th>Growth</th>
<th>Sporulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melampsora lini</td>
<td>A</td>
<td>45</td>
<td>0</td>
<td>4.6</td>
<td>3.3</td>
<td>Luxuriant</td>
<td>Frequent</td>
<td></td>
</tr>
<tr>
<td>Race 3</td>
<td>A</td>
<td>45</td>
<td>0</td>
<td>4.6 (or 2.3)</td>
<td>0</td>
<td>Luxuriant</td>
<td>Frequent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>45</td>
<td>0</td>
<td>4.6</td>
<td>3.3</td>
<td>Luxuriant</td>
<td>Infrequent</td>
<td></td>
</tr>
<tr>
<td>Puccinia graminis tritici</td>
<td>B</td>
<td>45</td>
<td>0</td>
<td>4.6 (or 2.3)</td>
<td>0</td>
<td>Luxuriant</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Race 126-ANZ 6, 7</td>
<td>C</td>
<td>45</td>
<td>0</td>
<td>4.6</td>
<td>3.3</td>
<td>Luxuriant</td>
<td>Infrequent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>45</td>
<td>0</td>
<td>4.6</td>
<td>3.3</td>
<td>Luxuriant</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Race 15B-4 and Race 56</td>
<td>C</td>
<td>45</td>
<td>0</td>
<td>4.6</td>
<td>3.3</td>
<td>Luxuriant</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

*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-): medium A: Sucrose, 50 g; Ca(NO₃)₂·4H₂O, 2.0 g; KNO₃, 0.25 g; MgSO₄·7H₂O, 0.25 g; KH₂PO₄, 0.25 g; K,HPO₄, 0.25 g; NH₄NO₃, 42 mg; 0.8 ml micronutrient solution (containing 13% NaFe (Geyig)), 10 g; MnSO₄·7H₂O, 447 mg; KI, 10 mg; NiCl₂·6H₂O, 18 mg; Ti(SO₄)₂·9H₂O, 42 mg; ZnSO₄, 35 mg; CuSO₄·5H₂O, 15 mg; BeSO₄, 20 mg; H₂PO₄ (85%), 10 mg; H₂SO₄ (concentrated), 0.2 ml; all in 200 ml glass distilled water. Medium B: glucose, 30 g; NaNO₃, 2.0 g; KCl, 0.5 g; MgSO₄·7H₂O, 0.5 g; KH₂PO₄, 1.0 g; Ca(NO₃)₂·4H₂O, 2 to 6 g; FeSO₄·7H₂O, 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.

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. 1a 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. 1c 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 prospores. 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 126ANZ 6.7. This, however, resembled race 3 of M. lini in its requirement for amino acids. Two North American isolates, race 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. 1e and...
Table 1). When glutamine was replaced with aspartic acid after 3 weeks' growth, vegetative growth continued and the stroma 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. 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. Immunologically specific suppression of GVH reactions has been achieved by immunisation of F1 hybrid host to produce antibody reactive with receptors on donor lymphoid cells for host antigens.

The GVH reactivity of lymphoid cells from the appropriate parental strain was suppressed in F1 hybrids producing such anti-receptor antibody (ARA). 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 F1 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) F1 hybrid rats either each received 400 rad of total-body X irradiation or were left untreated. Twenty-four hours later each group received from 2.7×10⁴ to 2×10⁵ 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 10⁵ 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 10⁵ 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 2×10⁵ survived. All irradiated rats receiving more than 1×10⁵ 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 chemoattractant factor (product of antigen recognition or PAR) generated when parental lymphoid cells are cultured briefly with hybrid lymphoid cells. The other method involves measurement of the interaction between the specific alloantibody used for immunisation and serum from the immunised hybrid animal. Such interaction can be detected by precipitin reactions in gel. 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 rats 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 F1 hybrid rats were given 400 rad of total-body X irradiation or were left untreated. Twenty-four hours later, 5×10⁴ or 1×10⁵ Lewis spleen cells were injected into each rat.
Table 1  Development of ARA in F₁ hybrid rats receiving parental strain normal Lewis spleen cells

<table>
<thead>
<tr>
<th>ARA titre*</th>
<th>Days after parental strain spleen cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 14 21 28† 35 42</td>
</tr>
<tr>
<td>5 × 10⁷ Lewis spleen cells</td>
<td>0 0 0 0 64 4 0</td>
</tr>
<tr>
<td></td>
<td>0 0 32 512 128 64</td>
</tr>
<tr>
<td></td>
<td>0 0 16 256 32 0</td>
</tr>
<tr>
<td></td>
<td>0 0 32 64 0 0</td>
</tr>
<tr>
<td></td>
<td>0 0 8 16 0 0</td>
</tr>
<tr>
<td>1 × 10⁷ Lewis spleen cells</td>
<td>0 0 128 256 Not done Not done</td>
</tr>
<tr>
<td></td>
<td>0 0 32 64</td>
</tr>
<tr>
<td></td>
<td>0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>0 0 8 4</td>
</tr>
<tr>
<td></td>
<td>0 0 256 64</td>
</tr>
<tr>
<td>400 rad plus 5 × 10⁷ Lewis spleen cells</td>
<td>0 0 Dead</td>
</tr>
<tr>
<td></td>
<td>0 0 Dead</td>
</tr>
<tr>
<td></td>
<td>0 0 0 0 Dead</td>
</tr>
<tr>
<td>400 rad plus 1 × 10⁷ Lewis spleen cells</td>
<td>0 0 Dead</td>
</tr>
<tr>
<td></td>
<td>0 0 Dead</td>
</tr>
<tr>
<td></td>
<td>0 0 0 0 Dead</td>
</tr>
</tbody>
</table>

* 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.

Against saline, the IgG antibody was coupled to sheep erythrocytes by means of chronic chloride. 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 titred 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.

As observed previously, all irradiated hybrid rats receiving 5 × 10⁷ Lewis spleen cells and four or five irradiated rats receiving 1 × 10⁷ Lewis spleen cells were dead within 24 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 anti-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.

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: 10⁷ Lewis spleen cells; 10⁷ Lewis spleen cells plus 5 × 10⁷ normal LBN spleen cells, or 10⁷ Lewis spleen cells plus 5 × 10⁷ 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 to produce GvH reactions. Recent evidence obtained from GvH reactions in vivo and mixed lymphocyte cultures in vitro 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. Cytoto-

Fig. 2  Protection of irradiated LBN F₁ rats from lethal GvH disease by adaptive transfer of F₁ spleen cells. Irradiated LBN rats receiving 1 × 10⁷ normal Lewis spleen cells only (——) all died of GvH disease. Irradiated LBN rats receiving 1 × 10⁷ normal Lewis spleen cells and 5 × 10⁷ spleen cells from F₁ rats resistant to GvH disease (-----) suffered no mortality or weight loss. The group of irradiated LBN rats receiving 1 × 10⁷ normal Lewis spleen cells and 5 × 10⁷ 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.
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 basophile leukocytes1,2. 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-ones3 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.

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 azaprinone was prepared by nitrous acid treatment of the diamine (2) which itself was prepared from O-propoxybenzamide 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 vitro4, second, the inhibition of a reagin-mediated PCA reaction in the rat5 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 azaprinone.

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
of 0.1 mg kg⁻¹ of M&B 22,948 and 2–5 mg kg⁻¹ of disodium cromoglicate. Intravenous theophylline had approximately one-fiftieth the potency of disodium cromoglicate.

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 cromoglicate 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 cromoglicate (100 mg kg⁻¹ intravenously) produced a protection comparable to that of 10 mg kg⁻¹ of disodium 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 cromoglicate, 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 spasmodenic effects of histamine, SRS-A and PGE₂ 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 intradural 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).

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; PGE₂, 15; PGF₂α, 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 any 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-

---

**Table 1**

<table>
<thead>
<tr>
<th>Route of drug administration</th>
<th>Dose (mg kg⁻¹)</th>
<th>Time (min) between dose and bronchial allergy challenge</th>
<th>Mean protection %</th>
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<tbody>
<tr>
<td>Intravenous</td>
<td>5</td>
<td>1</td>
<td>19 (4)†</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1</td>
<td>50 (4)</td>
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<tr>
<td></td>
<td>20</td>
<td>1</td>
<td>69 (4)</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>1</td>
<td>40 (4)</td>
</tr>
<tr>
<td>Oral</td>
<td>200</td>
<td>1</td>
<td>48 (6)</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>1</td>
<td>43 (3)</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>1</td>
<td>42 (3)</td>
</tr>
<tr>
<td>Inhalation</td>
<td>50</td>
<td>1</td>
<td>34 (11)</td>
</tr>
<tr>
<td></td>
<td>(mg ml⁻¹)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.
spasm in the guinea pig. The potency of M&B 22,948 was up to 50 times that of disodium cromoglicate, 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 oxanthone derivative (AH 7725) in inhibiting an allergen-induced immediate-type asthmatic response in man.

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Significance of immunofluorescent staining of lymphocytes with antisera to IgM immunoglobulins

IMMUNOLOGICAL reactions depend on 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 8 and rosette formation with red cells sensitised with some components of complement 8. Since the cells that show surface immunofluorescence are retained on columns of beads coated with antibodies to immunoglobulins, surface fluorescence is generally interpreted to mean that immunoglobulins are present on the surface of B lymphocytes.

Various means have been devised to prove this point primarily by using high resolution optical devices 9 or specific antisera 8. 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 8,9 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 immunoglobulin, on their surface. We were therefore surprised to find that it was possible to quantify IgG separable from human B cells 8 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 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 antisera 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

Fig. 1 Double diffusion in agar of IgM immunoglobulin (A) with anti-CHO (C), anti-α (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 on a column 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 pH 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 30 ml h⁻¹. The fractions reacting with an antisera 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 antisera 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 solute containing IgM immunoglobulin frozen. The purified IgM was digested first with trypsin (1%, w/w) at pH 8.0 in 0.65 M Tris HCl containing 0.01 M CaCl₂ 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 x 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 antiseraum (C above) is specific for these five oligopeptides.
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 anti-serum to human IgM.

Proteolysis of IgM with trypsin and pronase has been shown to degrade extensively both μ and L chains of IgM. 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 tryptic-proteolysate 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 polyepitope chain, representing the non-reducing carbohydrate ends. These five glycopeptides comprise all the carbohydrate of the μ chain. Purified IgM was digested with trypsin followed by pronase. The glycopeptides obtained after hydrolysis were separated on Sephadex into Mehlish-positive materials that on paper chromatography (butanol:ethanol:acetic acid-water:water 2: 1: 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 method of the axen and Ernhack. To assure that the carbohydrate moiety of the glycopeptides would bind to the immunoadsorbent, Sepharose 4B activated with CNBr was coupled to 1–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 antisera to normal human IgM globulin. The antisera was rendered non-specific for the μ chains of IgM by passage through immunoadsorbents made by coupling human IgG and IgA immunoglobulins and Bence Jones proteins to Sepharose 4B. This rabbit IgG globulin (1 g) was incubated at 37°C for 2 h with the immunoadsorbent containing the glycopeptides of IgM in phosphate-buffered saline (PBS) at pH 7.2. The unbound globulin was eluted at room temperature, and the absorbent washed with PBS.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Percentage of cells staining with fluorescein-labelled antisera to human IgG and IgM immunoglobulins and the glycopeptides (CHO) or polypeptide chains (μ) of IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>Pre-incubation of Cells with Antiserum to antigen (% of cells staining)</td>
</tr>
<tr>
<td>B1*</td>
<td>CHO</td>
</tr>
<tr>
<td>B1</td>
<td>CHO</td>
</tr>
<tr>
<td>B1</td>
<td>IgM</td>
</tr>
<tr>
<td>B1</td>
<td>μ</td>
</tr>
<tr>
<td>B2+</td>
<td>CHO</td>
</tr>
<tr>
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<td>CHO</td>
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<td>IgM</td>
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<td>μ</td>
</tr>
<tr>
<td>BM+</td>
<td>IgM</td>
</tr>
<tr>
<td>BM</td>
<td>CHO</td>
</tr>
</tbody>
</table>

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-1N HCl 2: 1: 1: 1: 1 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^-4 mol 1^-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).

Bound antibodies were eluted with a glycine/HCl buffer, pH 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 precipitation against IgM. Immunodiffusion in agarose (I.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 antisera. 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 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. 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 EA.C142. 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 IgM in vitro. The second cell population comprised small cells with a high density which sedimented toward the bottom of the gradient (20, 51%; albumin, B2), and these could be induced to form IgM 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 reacted with fluorescein-labelled 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 unchanged when all 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, surface and surface fluorescence. The cytoplasmic fluorescence of the plasma cytoplasmoid 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 antisera had activity against the μ chain determinants of IgM. This activity could be inhibited by preincubating the antisera with IgM but not with the glycopeptides of IgM, showing specificity of this antisera 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 antisera to IgM or to the glycopeptides in a pattern showing surface fluorescence. This activity could be inhibited by preincubating the antisera with the glycopeptides but with IgM showing specificity of the antisera 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 antisera (5 ml) was adsorbed 10 times with 10^6 B1 cells each time at 37°C for 1 h. The adsorbed antisera 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 antisera with both B1 and B2 cell populations.
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.

We thank Dr Chester Alper for the gift of the anti-IgM antiserum and Dr Harvey Colten for providing the sheep red cell intermediate. This investigation was supported by a United States Public Health Service Grant. J.G. is the recipient of a Queen Elizabeth II grant from the Canadian Government.

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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 autogenous 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 or in vitro coating of antigenic determinants on cancer cells.

Some studies have shown that it is possible to increase the immunogenicity of tumour cells. In our laboratory immunogenic L1210 sublines were induced by prolonged in vivo treatment with the drug 5-(3,3-dimethyl-triazeno)imidazol-4-carboxamide (DIC). 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.

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 CDF₁ 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 ⁵¹Cr release assay, modified by Canty.

A sample of 10⁷ 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 × 10⁷ (50 μl) ⁵¹Cr-labelled cells (targets) on a rocking platform at 37°C in a moist atmosphere of 10% CO₂–90% air. Radioactivity released in the supernatant by lysis of target cells was counted in an automatic scintillation counter.

In each experiment triplicated 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:

\[ \text{c.p.m.} \frac{\text{⁵¹Cr released from incubated cells}}{\text{c.p.m.} \frac{\text{⁵¹Cr released from frozen-thawed cells (4 times)}} {\times 100}} \]

Experimental results were expressed as (% cytotoxicity in experimental samples)—cytotoxicity in control samples. The ⁵¹Cr release in control samples was 12 ± 3%.

Spleen cells from mice immune to L5178Y/DIC tumour cells were highly cytotoxic in vitro to ⁵¹Cr-labelled L5178Y/DIC cells. Parental radio-labelled L5178Y cells and other tumour and thymus cells syngeneic or allogeneic with CDF₁ mice, did not release the isotope after incubation with anti-L5178Y/DIC attacker cells. The specific cell-mediated ⁵¹Cr release indicates

![Fig. 1](image)

Fig. 1: Kinetics of immune spleen cell activity. ——, Direct assay; ——, assay 24 h after incubation. Attacker cells were from CDF₁ mice immunised with 10⁷ L5178Y/DIC viable cells injected i.p. and assayed against ⁵¹Cr-labelled L5178Y/DIC cells. In these and the following assays the ratio of attacker cells to labelled target cells was 200:1.

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Table 1  Cytotoxic activity of spleen cells immunised against algogenic antigens

Attacker cells | Target cells | $^{51}$Cr release (± s.e.)
---|---|---
EL$_4$ | L5178Y | 14.1 (0.4)
EL$_4$ | L5178Y/DIC | 2.8 (0.6)
EL$_4$ | E$_{G_4}$ | 16.6 (0.8)
C$_{(57BL/10)SCr}$ | L5178Y | 3.4 (1.1)
C$_{(57BL/10)SCr}$ | L5178Y/DIC | 2.8 (0.8)
C$_{(57BL/10)SCr}$ | C$_{(57BL/10)2Cr}$ | 42.8 (0.7)
C$_{(57BL/10)2Cr}$ | L5178Y | 3.3 (0.9)
C$_{(57BL/10)2Cr}$ | L5178Y/DIC | 1.8 (0.9)
C$_{(57BL/10)2Cr}$ | C$_{(57BL/10)2Cr}$ | 12.4 (1.1)
C$_{(57BL/10)2Cr}$ | L5178Y | 2.0 (0.6)
C$_{(57BL/10)2Cr}$ | L5178Y/DIC | 3.5 (0.8)

Attacker cells (S) were spleen cells from CDF$_1$ mice 10 d after immunisation with an i.p. inoculum of 10$^5$ tumour cells containing 10$^5$ E$_{G_4}$ or an SC implant of 1 mm$^3$ skin tissue pieces (C$_{(57BL/10)SCr}$ or C$_{(57BL/10)2Cr}$). L5178Y and L1210 were from Microbiological Ass. Bethesda. EL$_4$ and E$_{G_4}$ were obtained from Dr. G. Della Porta, National Cancer Institute, Milan. Moloney Sarcoma (MSV) and Gross leukaemia (GLV) were virus-induced by Dr A. Di Marco, National Cancer Institute, 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.

Immune spleen cells from CDF$_1$ mice sensitised to algogenic 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 any nonspecific immunosensitivity. Evidently neither L5178Y nor L5178Y/DIC associated antigens can react with $H-2^a$- 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 L5178Y/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).

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).

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.

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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, using
<table>
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<tr>
<th>Inhibitor (µg/0.2 ml)</th>
<th>Lymphocytes (PHA(H-Thymidine Mikro) (Ly-control)</th>
<th>Inhibitor (µg/0.2 ml)</th>
<th>Lymphocytes (MLC(H-Thymidine Mikro) (Ly-control)</th>
<th>Inhibitor (µg/0.2 ml)</th>
<th>HeLa (H-Thymidine)</th>
<th>HeLa (H-Thymidine)</th>
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different preparations of liver antigen from human liver, led us to attempt to prepare a better defined antigen from human liver—already proposed for this test system. Besides a liver specific protein as previously described, 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 100,000g for 90 min in Hanks solution, pH 7.2. The supernatant was transferred to a Sephadex G-100 column, equilibrated with 0.01 M Tris-HCl buffer, pH 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 × 10^4-8 × 10^6. 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 phytohaemagglutinin (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 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 lymphocytes 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, 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 Diemctus viridescens 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 Decker. 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, because chalones are defined as non species-specific inhibitors, present in and produced by the tissue, the proliferation of which they specifically inhibit.

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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3 Schumacher, K., and Alzer, G., Die chronische Hepatitis (edit. by Lindner, H.), (Gerhard Wittstock, Baden-Baden-Brüssel)

Two types of resistance to polycene antibiotics in Candida albicans

STUDIES reported during the past 15 years have led to the widely accepted conclusion that polynene antibiotics function
by binding with the sterol molecules in susceptible cells to cause permeability change and eventual death of the cell in recent reports based on liposomal model membrane studies, it has been suggested that susceptibility to polycene antibiotics is determined by organisation rather than by a single component of the membrane, namely steryl. Here, through study of polycene-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 polycene antibiotics.

Five mutagen-induced mutants were derived from a clinical isolate of Candida albicans (E139-A, wild type) as described by Hamilton-Miller. 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 Liebmann–Burchard colorimetric reaction (Table 1) and, in accordance with previous reports, contained proportionally more sterol (Table 2). In other laboratories, differences in the sterol pattern of polycene-resistant mutants have been observed, and the major sterols have been characterised completely in one set of mutants. Polycene resistance was attributed to altered patterns of sterols. In several studies, decrease in ergosterol content has been singled out as the direct cause of polycene resistance. For comparison, the ultraviolet absorption spectra of the non-saponifiable fraction extracted from E139-A and our polycene-resistant mutants were examined. 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.

We have found that effects on viability and cell leakage depend on temperature and phase of growth. At room temperature (23°C–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
Table 1 Characterisation of polyene-resistant mutants of *C. albicans*

<table>
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<tr>
<th>Polyene added (µg ml(^{-1}))</th>
<th>Strain</th>
<th>E139-A</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A7</th>
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<td>Growth in liquid medium*</td>
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<td>2.5</td>
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<tr>
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<td>Colour reaction†</td>
<td>Amphotericin B</td>
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</tr>
</tbody>
</table>

| Doubling time (min)§           | Greenish blue | 65 | Bright yellow | 85 | Purplish Blue | 70 | Bright Yellow | 97 | Greyish Blue | 90 | Greyish blue | 135 |

* Log phase cells were adjusted to approximately 5 x 10⁴ 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 hr of incubation at 37°C. The nystatin (4530 µl ml⁻¹) and the amphotericin B (876 µg 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 effects 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); (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, protonome (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. 1a) and A7 (Fig. 1e). 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.
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. 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. 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 buffer to about pH 7) for various times. The reaction was terminated by addition of 10⁻³ to 10⁻⁴ M Nα-Tosyl-L-lysine chloromethyl ketone (TLCK) and the fibrinogen was dialysed to low ionic strength (5 x 10⁻³ M KSCN, 0.01M 2-(N-morpholino)ethane sulphonic acid (MES), pH 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 stage, 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. 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. 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)

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. Following a similar approach, we have crystallised fibrinogen. Native fibrinogen precipitated at low ionic strength forms
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 clottability 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 P2₁, 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. 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, 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. Although these large periodocities 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.

This work was supported by grants from the US Public Health Service and the National Science Foundation. We thank Dr Stephen Harrison for taking the X-ray photographs and for discussions. We thank Dr D. L. D. Caspar for discussions, Isa Bernardini for technical aid and Paul Norton for electron microscopy.

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Received May 2; revised August 2, 1974.

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**64-78, Kingsway, LONDON, WC1B 6BD**

by the 30th November, 1974

Applications in U.S.A. and Canada should be sent to

**The Counsellor (Scientific),**

**Embassy of Australia, 1601 Massachusetts Avenue, N.W., WASHINGTON, D.C. 20036, U.S.A.**

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The University is now considering long-range plans for expansion of its teaching and research capability in Fisheries Biology. A major programme of fishery and environmental research of fjords is being developed by a group of biologists led by Professor A. H. Weatherley. Other fishery research programmes are being planned.

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(1466)
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[1493]

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[1532]

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[1483]

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Further particulars are available from the Secretary-General, Association of Commonwealth Universities (Apcis), 36 Gordon Square, London WC1H 0AA, or from the Registrar of the University.

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[1488]

ACADEMIC POSITION

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[1489]

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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 £2,407. Applications, giving the names of three referees, should be sent to Professor A. G. Ward, Keeper 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 £19,618 per annum and for an Associate Professor is £16,389 per annum.

A statement of Conditions of Appointment and Information for Candidates may be obtained either from the Secretary-General, Association of Commonwealth Universities (Apcis), 36 Gordon Square, London WC1H 0AA, or from the Registrar, University of Sydney, N.S.W. 2006, with whom applications close on November 29, 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]
SCIENCE 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, coordination 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)
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.D.s. 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. Burston 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 fortran 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 Burston 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)

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 farm 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:

J. F. Pattison, Employment Manager,

The Boots Company Ltd.,
Station Street, Nottingham NG2 3AA.
Tel: Nottingham 5625 Ext. 243.

(1510)

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 to be taken 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

Electrical Engineering

Civil Engineering
Structural Design and Analysis
Seal and Foundation Engineering
Hydraulics and Hydrology
Environmental Engineering

SALARY: Senior Lecturer—RM51,405 by 1.735% to RM61,350 (Basic) Lecturer—RM39,400 by 1.735% to RM47,125 by 1.735% to RM55,000 (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 RM51,405 per month, and, in case of appointment 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 from 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 (Appia), 36 Gordon Square, London WC1H 0FF, or obtained directly from:

The Registrar,
National Institute of Technology,
Kuala Lumpur (15-02),
Malaysia.

(1494)

THE MIDLAND CENTRE FOR NEUROSURGERY AND NEUROLOGY
Holly Lane, Smethwick.
Warley, Worecs. 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 A.I.M.H. 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 cooperation 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 cooperation 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.
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 Pharmacology could be an advantage.

Further details and application forms from Dr R. E. Yorke, Department of Science, Oxford Polytechnic, Oxford. OX3 0BP.

(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 nomenclature is essential and experience of automation would be an advantage. Salary on Whitely Council Scales. Applications and enquiries to the Director, North London Blood Transfusion Centre, Deanstreet Road, Edgware, Middlesex.

(1538)

POSTDOCTORAL RESEARCH

Mass Spectrometry ion source research on isolation 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, A/E, 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 located 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 palaeobotanical and microfossil stratigraphical and mineralogical correlation methods to mid-precambrian stratigraphy and mineral exploration.

Candidiates 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 authorized 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 Cyanium oxalate with organic and natural biological tissue and membrane materials. Tenure 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 is desirable. Applicants should submit a 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 laboratory. Previous experience together with I.S.C. & C. Science Laboratory Technician or the equivalent, H.N.C. or similar qualification. The post will be Salary £2,007 by £2,050 p.a., but if candidate does not have the appropriate experience, qualifications appointment would be at the Grade 3 salary scale £1,960 by £2,150 p.a. In both cases, in addition £200 p.a. London Weighting and current threshold payments. Five day week. Five weeks (total) annual leave. Pension Scheme. Letters only to Assistant Secretary (Establishments) F/Ph. 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 problems related to milk processing. A candidate with ambition is needed who preferably also has an interest in physiological or molecular chemistry, with a minimum qualification of B.Sc. or equivalent. Salary according to qualifications and experience. Appointments are renewable annually.

(A1968)
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 freshwater 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 799/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, LARAZ, 1 avenue de Béjar, 1120, Brussels, Belgium.

(1520)

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 £1,500 to £2,265 (inclusive of London allowances) plus supernumerary with F.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 research in histology, autoradiography, and electron microscopy, with support from technicians.

This is a well-equipped laboratory and the successful candidate will be expected to have a background in the field of science and be able to take on administrative responsibilities. Written applications giving full curriculum vitae and references to Laboratory Superintendent, Kennedy Institute of Rheumatology, Bute Gardens, London W6 7DW, by November 9, 1974.

(1507)

UNIVERSITY OF ABERDEEN

SENIOR LECTURSHIP IN MEDICAL PHYSICS

Applications are invited from experienced radiological physicists to be responsible for teaching in medical physics to medical students and for other duties as may be assigned. Applications should be accompanied by a curriculum vitae and should be submitted to 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 (around £3700 ~ £4700)
- Ref: SB/11/AE.

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 ~ £4700), Higher Scientific Officer (£2700 ~ £3600), or Scientific Officer (around £1850 ~ £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 ~ £2900).
- Application forms (for return by 6 November 1974), from Civil Service Commission, Aken Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222 ext. 300 or, (for 24 hour answering service), London 01-839 1992.

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 contaminants 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 ~ £4800)
- Ref: SB/12/AE.
- Application forms (for return by 7 November 1974), from Civil Service Commission, Aken Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222 ext. 300 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 ~ £4600)
- Ref: SB/18/AD.
- Application forms (for return by 8 November 1974), from Civil Service Commission, Aken Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222 ext. 300 or, (for 24 hour answering service), London 01-839 1992.

Science Group
Civil Service
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 of nitrate 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 travel ■ Age under 27 ■ 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 estuaries, 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.
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- In Marine Environment Protection Division ■ Examine samples of marine sediments from estuaries, 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
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

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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.
UNIVERSITY OF THE WEST INDIES — TRINIDAD
Applications are invited for:
(a) SENIOR LECTURESHIP

(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$40,010 to TT$52,730 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 passage and 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.

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 (order review): Lc2-400 to Lc4-740 p.a. (€1 sterling=Lc2). The British Government may supplement salary in range £750 to £1,300 p.a. (stipend) for married appointee or £350 to £700 p.a. (stipend) for single appointee, normally for a maximum period of 2 years. Appointee will be granted the necessary leave for field work and conference attendance, and will be entitled to 3 months’ leave in the second year. Applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent to the Secretary, University of Sierra Leone, P.O. Box 174, Freetown, Sierra Leone. Applications 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.

ROYAL POSTGRADUATE MEDICAL SCHOOL
POSTDOCTORAL CHEMIST OR BIOCHEMIST
required for Department of Clinical Pharmacology to undertake studies of drug metabolism in animals and man. Applicants 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., Hamмermith Hospital, Queen’s Gate Road, London W12 0HS, quoting Ref. No. 20/487/N.

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 tonic crops.
The project is funded over a 5 year period by the Wolfson Foundation, and the successful candidates will be attached to the Department of Agriculture, or of Agricultural Biology according to research and experience.
Candidates should have postgraduate experience preferably a Ph.D. in Applied Plant Genetics, plant breeding, experimental agronomy, and must have a special concern for the practical application of the research programme.
Salary in range £2,605 to £2,602 including F.S.S.U. The appointment will be initial for three years with possibility of extension to a maximum of 5 years.
Further details may be obtained from Professor A. White, Wolfson Laboratories, University of Reading, Whiteknights, Reading RG6 2AS, to whom applications including the names of two referees should be sent.

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, 1973. 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 range negotiable and to be commensurate with training and experience. Applicants should direct their correspondence to Dr. James T. Gillette, Chairman Search Committee, New York State Veterinary College, Cornell University, Ithaca, N.Y. 14853.

The New York State Veterinary College is an Equal Opportunity Affirmative Action Employer.

Senior CNS Pharmacologist
Lilly Research Centre
Surrey

We seek a broadly-based Neuropsychopharmacologist or Neuropharmacologist to lead a newly created team of eight qualified and technical staff conducting fundamental research on neuroleptic and antidepressant drugs.
The Lilly Research Centre is one of the largest 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 anxiety, inflammatory disease and disorders of the central nervous system. The CNS research programme has particular emphasis on behavioural pharmacology, CNS biochemistry and neurophysiology.

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 neuropsychopharmacology. 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

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 research on steroids for the Collection. Much of this work involves research into new methods of synthesis, or the synthesis of novel steroids. Sufficiently qualified graduates may be permitted to undertake postgraduate studies for the M.Phil. or Ph.D. degree. Salary: Postdoctoral scale £2,118 to £2,412 plus threshold and London Weighting (under review) plus threshold. Postgraduate student, equivalent to an M.R.C. student (under review).

Applications, including the names of two referees, should be addressed to Dr. D. N. Kirk, Chemistry Department, Westfield College N., Kidzenger Avenue, Hampstead, London NW3 2ST. (1534)

UNIVERSITY OF PAPUA NEW GUINEA
(Port Moresby)

Applications are invited for the post of CHIEF CARTOGRAPHER, E.M.H., in the Department of Geography. Applicants must be experienced Cartographers. Appointments will be responsible for the supervision of junior cartographers, and will be required to prepare maps for research, publications and classes. A knowledge of photigraphic and lithographic reproduction as well as basic photogrammetric procedures would be an advantage. The Department of Geography possesses a standard cartographic 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: AS$9,099 to AS$10,432 p.a. plus AS$300 p.a. dependants allowance, if married AS$500 p.a. dependants allowance if single. (These salaries include an overseas allowance). (£1 sterling = AS$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 house fairs. 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 references, a recent small photograph and an indication of estimated starting date. Applications should be sent to K. R. Long, Secretary, P.O. Box 4620, University, Papua New Guinea. Closing date: November 13, 1974. (1527)

THE UNIVERSITY OF LIVERPOOL
SUB-DPARTMENT 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 Bradly,
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 computerized 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 addressed to 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 £5100 - £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 abnormalities, 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).
Applicants (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.


(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 R. N. Bunker. Salary in the range £2,118 to £2,580 p.a. plus threshold payments and participation in F.S.S. 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.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

available 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 SMITSON FELLOWSHIPS which are restricted to students of Philosophy or Psychology but is open to women graduates of any University.

Unless she chooses otherwise, a Research Fellow who has not yet obtained a Ph.D. will receive £998 a year and free board and residence. If she chooses residence, she will receive £1,230 a year. For a Research Fellow who has already obtained a Ph.D. degree, the entitlements 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.

(1350)

GIRTON COLLEGE
CAMBRIDGE

A Clothworkers' Company Visiting Fellowship is available for a senior woman scientist for the academic 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 the development of a novel small rechargeable battery containing solely organic constituents. A specialised knowledge of electrochemistry is not required. Prof. W. A. Hofmann organically 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 master's degree or a lower second degree with several years relevant industrial experience. The grant is £800 p.a. tax free; this would be doubled 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 141, Leicester LE1 9BH. (1472)

UNIVERSITY OF READING
NATIONAL COLLEGE OF FOOD TECHNOLOGY
Applications are invited for a 3 year S.R.C. (C.A.E.S.) 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, Department of Sciences in Technology, with a biological background, with some knowledge of Microbiology and Biochemistry preferred. Applications and names of two referees should be sent 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 for a studentship in the area of research in medical sciences for a student to join a group working on several aspects of autonomic nervous control of the cardiovascular system. Applicants should have a good second class honours degree or equivalent in a biological science. Applications should be made before 15 October 1974. (Ref: MR 50) (1518)

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 Anthostomus; 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 (38,700 per annum). Application with curriculum vitae and names of two referees to: K. J. Stevenson Department of Chemistry Biochemistry Group University of Calgary Calgary, Alberta, Canada T2N 1N4 (1540)

SENIOR NICHOLAS FELLOW
required. The position would be renewable subject to completion of the three-year contract. Salary in the region of £15,000 per annum. Application should be made to Dr. D. L. Degwey, Laboratory, Mount Vernon Hospital, Northwood, Middlesex HA6 2RN. (1501)

LECTURES AND COURSES
UNIVERSITY OF LEICESTER
DEPARTMENT OF CHEMISTRY AND SCHOOL OF EDUCATION
Ph.D. IN CHEMISTRY WITH GRADUATE CERTIFICATE OF EDUCATION
Applications are invited from pure or holding, or expecting to be awarded during 1975, a degree with First or Second Class Honours (Upper division), or equivalent qualifications, in Chemistry, Combined Sciences, Chemical Engineering, Physics, etc., who wish to pursue a 3-year course of research and teacher training. The course may attract a limited number of S.R.C. research studentships, particularly for new graduates, but candidates from industry or teachers may be eligible to apply for awards under other S.R.C. schemes. For further information and application forms please write to Professor R. D. Peacock, Department of Chemistry, University of Leicester LE1 7RH. The closing date for applications is January 31, 1975. (1530)

GRANTS & SCHOLARSHIPS
FRENCH GOVERNMENT SCHOLARSHIPS
For further study and postgraduate research in France in Pure and Applied Science, Technology, Medicine, Social Sciences, Economics, Geography and Agriculture. Applications are invited for the academic year 1975-1976. Candidates must be French citizens, holders of a B.A. or an equivalent degree. Closing date: December 31, 1974. Further information and application forms from Scientific Department, French Embassy, 41 Parkside, Knightsbridge, London SW1X 7JF. (1522)

SYMPOSIUM
UNIVERSITY OF TEHRAN
TEHRAN, IRAN
ANNOUNCES AN INTERNATIONAL SYMPOSIUM to be held IN TEHRAN on MAY 5, 6 and 7, 1975
Sponsored by THE INTERNATIONAL UNION OF BIOCHEMISTRY, THE INTERNATIONAL UNION OF PURE AND APPLIED BIOPHYSICS ON The Structural Basis of Membrane Function with sessions on Membrane Structure, Transport in Membranes, Energy Transfer in Membranes, Regulatory Functions of Membranes, Excitable Membranes, and Microsomal Membranes. Information regarding Symposium Registration, Hotel Reservation, and Sight-seeing Excursion on May 8 and 9, etc., may be obtained from: DR. K. JAVAHERIAN POST OFFICE BOX 14-1700 UNIVERSITY OF TEHRAN TEHRAN, IRAN (1474)
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Molecular hybridization with 3H-labeled Polyuridylic acid (Poly U) has been used to detect Poly A sequences in viral RNA.


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Measures leaf area on living plants or detached leaves in the field or laboratory.

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