22 MAY 1979 (FOUO 15/79) 1 OF 1

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USSR

TRANSLATIONS ON USSR SCIENCE AND TECHNOLOGY

BIOMEDICAL AND BEHAVIORAL SCIENCES

(FOUO 15/79)

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TRANSLATIONS ON USSR SCIENCE AND TECHNOLOGY

BIOMEDICAL AND BEHAVIORAL SCIENCES

(FOUO 15/79)

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AGROTECHNOLOGY

UDC 633.511:546.135+546.46

EFFECT OF DEFOLIANTS ON THE OPENING OF COTTON BOLLS

Moscow DOKLADY VSESOYUZNOY ORDENA LENINA AKADEMII SEL'SKOKHOZYAYSTVENNYKH NAUK IMENI V. I. LENINA in Russian No 2, 1979 p 9

[Article by Cand Ag Sci A. Zhurakulov and A. I. Imamaliyev, Academician of the All-Union Order of Lenin Academy of Argicultural Sciences Imeni V. I. Lenin]

[Text] It is known that the time it takes for cotton bolls to develop and open depends on the cotton variety as well as on the environmental conditions and the agricultural procedures employed. Boll development averages 55-65 days for Soviet varieties of long-staple cotton (4), and even longer for thin-staple varieties. In this case a significant proportion of the bolls open late and suffer exposure to autumn freezes, which reduces the quality and size of the yield and lengthens the picking time. As a result the time of autumn plowing comes later, which has an unfavorable effect on the productivity of the following cotton or other crop. Better conditions are created for opening of the bolls by defoliation and removal of leaves. In this case the air temperature around the plants increases by 4.9-6.1°C in comparison with control, while on the other hand relative air humdity declines by 8.4-10 percent (3). However, this agricultural procedure does not always accelerate maturation and opening of the bolls (2). With early defoliation (in the last third of August), transformation of soluble carbohydrates into cellulose is significantly inhibited in the fiber of young bolls, this being the cause of their slow opening (1). In this connection we studied the defoliation times of cotton varieties Tashkent 1, Tashkent 3, and Tashkent 4. The plants were defoliated with magnesium chlorate ($Mg(CeO_3)_2 \cdot 6H_2O$) at a dose of 12-15 kg/ha and butyphos ($(C_4H_9S)_3PO$) at a dose of 2.5-3.0 kg/ha. Plants processed with water served as a control. The experimental replication was fourfold; plot area was 60-120 m^2, and there five test rows 20-40 meters long in each.

The research results showed that the defoliants had a varying influence on maturation and opening of bolls depending on processing time and the chemical nature of the defoliant.

Growth in Percentage of Open Bolls of Tashkent Cotton Varieties
and Difference with Respect to Control
on the 18th Day After Processing

(1) Обработка при раскрытии коробочек	(2) Ташкент 1		Ташкент 3		Ташкент 4	
	(3) прирост	(4) разница	прирост	разница	прирост	разница
1972						
1—2	15,7		25,4		—	—
	23,0	7,3	29,2	3,8	—	—
	30,2	14,5	35,8	10,4	—	—
2—3	16,9		18,7		—	—
	31,3	14,4	38,1	19,4	—	—
	43,1	26,2	46,7	28,0	—	—
1973						
2—3	18,1				17,5	
	37,1	19,0	—	—	34,5	17,0
	46,1	28,0	—	—	40,6	23,1
3—4	23,4		21,0		25,3	
	41,2	17,8	35,6	14,6	40,4	15,1
	48,0	24,6	42,4	21,4	47,5	22,2
1974						
2—3	20,7		28,9		12,2	
	39,2	18,5	56,6	27,7	22,5	10,3
	48,0	27,3	57,4	28,5	32,1	19,9
4—5	19,7		22,3		21,0	
	33,1	13,4	40,7	18,4	36,0	15,0
	37,0	17,3	43,1	20,8	39,0	18,0

Note: First line of figures--control, second--butyphos, third--magnesium chlorate.

Key:

1. Processed after opening of __ bolls
2. Tashkent
3. Growth
4. Difference

When one or two bolls were open, defoliation weakly stimulated their maturation and opening, the effect being stronger when 2-3 and 3-4 were opened.

With later defoliation, performed when 4-5 bolls were open, the rate of growth in the number of open bolls decreases for all varieties in comparison with the earlier processing time. This is associated with the decrease in air temperature by this time.

We can see from the data in the table that the greatest growth in the number of open bolls is observed with magnesium chlorate defoliation in relation to all varieties and processing times. The reason for this lies in the fact that the preparation considerably dehydrates the boll glumes.

Thus the optimum time for defoliating Tashkent varieties of cotton is the period in which from 2-3 to 3-4 bolls are open on the bushes. Deposition of cellulose in fiber and of reserve nutrients in seeds basically ends by this time, and therefore defoliation produces the greatest impact.

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BIOCHEMISTRY

UDC: 616.45-001.1.3:576.8.094.7:577.161.3

REGULATORY EFFECT OF ALPHA TOCOPHEROL ON CONDUCTION OF BILAYER PHOSPHOLIPID MEMBRANES FORMED OF RAT BRAIN AND LIVER PHOSPHOLIPIDS IN THE PRESENCE OF STRESS

Moscow DOKLADY AKADEMII NAUK SSSR in Russian Vol 244, No 6, 1979 pp 1496-1499

[Article by M. I. Agadzhanov, S. A. Badzhinyan, K. G. Karagezyan and V. G. Mkhitaryan, Institute of Biochemistry, Armenian Academy of Sciences, Yerevan; Yerevan State Medical Institute and Yerevan Institute for Advanced Training of Physicians (presented by Academician Ye. M. Kreps 23 Nov 77), submitted 13 Apr 78]

[Text] In the presence of many pathological processes, impaired vital function of cells is based on increased ion permeability of cellular and subcellular membranes, which is determined, to some extent, by intensification of the process of lipid peroxidation [1]. The latter is associated with intensification of free radical processes inherent in different stress states [2]. For this reason, it was necessary to investigate changes in ion condition in the presence of body stress on model bilary phospholipid membranes (BPM). Burn trauma was used as the stress factor. At the same time, it was necessary to determine the effect of α-tocopherol on these processes.

The studies were conducted on female rats weighing 140-160 g. We produced 3b degree burns on 12-15% of the body surface in the region of the hind legs using water at a temperature of 80°, with 10-s exposure. We measured conduction of BPM formed from phospholipids of brain and liver tissues of animals sacrificed 1 h, 1, 3, 7 and 15 days after trauma. Vitamin E was given in the form of α-tocopheryl acetate (which is hydrolyzed in the body to α-tocopherol [3], intraperitoneally at the rate of 1 mg/kg body weight, immediately after the burn, then 3, 7 and 12 days later.

The phospholipid membranes we used were obtained by the method described by Mueller [4]. Phospholipids were extracted from rat brain and liver tissues according to Folch [5]. Bilayer membranes were formed from phospholipids dissolved in heptane in a concentration of 20 mg/mℓ. Electric readings were taken using the method described in [6] with a direct current electrometer in 0.1 M KCl solution at pH 3.0, 6.0, 7.4, 8.0 and 9.0. In order to determine the role of α-tocopherol in forming cell membranes, we added vitamin E in a dosage of 0.04 mmole/mℓ to BPM from phospholipids isolated from the organs of burned rats in in vitro experiments. We added methyl ether of oleic acid and cumyl hydroperoxide (0.05 mmole/mℓ of each) to BPM from phospholipids taken from organs of intact rats to confirm the peroxide mechanism of impairment of membrane permeability. All measurements were taken at a temperature of 26°C.

As can be seen, conduction of BPM of phospholipids obtained from the liver (Fiugre 1a) and brain (Figure 2a) of normal rats is rather low, and it depends on medium pH. It was demonstrated that the stress state induced by burn trauma is characterized by increased BPM conduction at all tested times. Medium pH had a significant influence on this process, maximum increase in conduction in the liver (Figure 1) being noted at pH 6.0 and 7.4, whereas in the brain (Figure 2) these changes were more marked mainly at pH 9.0. The above changes in BPM conduction were not demonstrable after giving burned animals vitamin E, particularly with regard to BPM of phospholipids isolated from brain tissue (Figure 2).

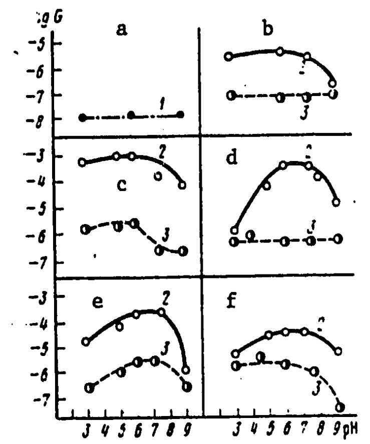

Figure 1.
Effect of α-tocopherol on conduction of BPM of rat liver phospholipids in 0.1 M KCl solution at different intervals after burn trauma. Y-axis, logarithms of specific membrane conduction log G ($ohm^{-1} \cdot cm^{-2}$)

a) normal
b) after 1 h
c) after 1 day
d) after 3 days
e) after 7 days
f) after 15 days
1) control
2) BPM conduction after burn
3) BPM conduction after burn + 1 mg/kg α-tocopherol

This regulatory effect of α-tocopherol is apparently related to its role in stabilizing cell membranes [7]. It is believed that vitamin E stabilizes membrane structure by means of specific physicochemical interaction between its lateral core chain and hydrophobic radicals of polyunsaturated phospholipid fatty acids, particularly derivatives of arachidonic acid. From this point of view, of interest are the data we obtained on quantitative levels of α-tocopherol and phospholipids in the brain and liver (Table 1) of rats who have sustained burned trauma. It was demonstrated that this is associated with a significant decrease in both α-tocopherol and total phospholipids of the brain and liver at all tested times. Interestingly enough, the decrease in phospholipids is related primarily to the fractions of lecithins and phosphatidyl ethanolamines. Concurrently, there is a decrease in degree of unsaturation of fatty acids in phospholipids, mainly due to polyene and, in particular, arachidonic acid (Table 2). Administration of α-tocopherol as described above normalizes the tested components.

Table 1. Effect of α-tocopherol on level of lipid peroxidation, endogenous α-tocopherol and phospholipid content of the rat brain and liver after burn trauma (% of control)*

Time after trauma, days	Factor	Lipid peroxides		Alpha tocopherol		Total phospholipids		Lecithins		Phosphatidyl ethanolamines	
		brain	liver	brain	liver	brain	liver	brain	liver	brain	liver
1 h	After burn	180	174	65	81	64	32	42	24	57	78
	Burn + α-Tp**	112	116	82	100	—	110	82	84	92	95
1	After burn	163	178	58	41	47	52	22	29	36	22
	Burn + α-Tp	118	102	79	200	126	132	79	72	94	112
3	After burn	147	128	75	73	58	54	30	40	43	28
	Burn + α-Tp	116	114	92	164	112	108	88	80	102	93
7	After burn	144	134	84	62	63	82	51	55	67	57
	Burn + α-Tp	118	105	—	100	131	108	80	63	109	82
15	After burn	189	126	109	59	43	48	17	26	28	36
	Burn + α-Tp	116	98	127	113	141	124	78	58	112	100

*The control is considered to constitute 100%.
**α-Tp--α-tocopheryl acetate (same in Table 2).

Table 2. Effect of tocopherol on arachidonic acid and phospholipid content of the rat brain and liver after burn trauma (%)

Time after trauma, days	Brain			Liver		
	control	after burn	burn + α-Tp	control	after burn	burn + α-Tp
—	14,57±0,12*			24,35±0,6*		
1 h		16,31±1,0	14,05±0,6		15,4±1.21	22,44±1,2
1		14,02±1,5	13,50±0,86		17,72±1,0	22,45±1,7
3		13,68±0,74	15,38±0,64		19,4±0,5	24,09±1,22
7		10,0±0,8	12,38±0,34		20,4±0,9	23,5±1,1
15		19,85±2,07	13,93±0,7		27,88±1,1	25,4±1,4

*Overall fatty acid content in indicated tissue is considered as 100%.

In in vitro experiments, we demonstrated that addition of 0.04 mmole/mℓ vitamin E to phospholipids from the liver of burned rats (burned for 1 h, pH 7.4, 0.1 M KCl solution) restores BPM conduction from $4.8 \cdot 10^{-6}$ $ohm^{-1} \cdot cm^{-2}$ to normal, i.e., to $8.3 \cdot 10^{-8}$ $ohm^{-1} \cdot cm^{-2}$.

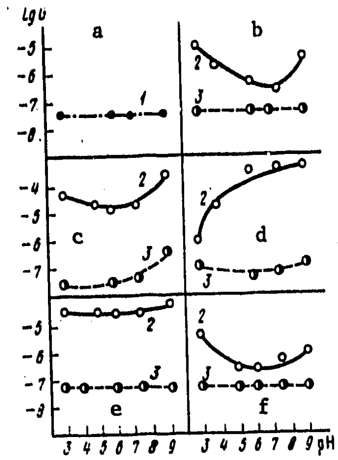

Figure 2.
Same as in Figure 1, but with BPM formed from phospholipids of the brain

The data we obtained are indicative of a certain parallel between BPM conduction, α-tocopherol and phospholipid content, as well as level of lipid peroxidation in rat brain and liver tissues (Table 1) after sustaining burn trauma. It was demonstrated that the burn also leads to significant increase in lipid peroxides immediately after trauma is sustained; a high level thereof persists for a long time (up to 30 days)[8]. Alpha tocopherol also normalizes the level of lipid peroxidation.

We added 0.05 mmole/mℓ oxidized methyl ether of oleic acid or cumyl hydroperoxide to phospholipids from the liver of intact rats in experiments in vitro, in order to prove the existence of a peroxide mechanism of impairment of membrane permeability in the presence of stress reactions. It was demonstrated that, in both instances, conduction of BPM increases by about a factor of 10^3. Analogous data were submitted in [1].

Thus, stress induces an increase in levels of lipid peroxide in the body, which intensify oxidation of α-tocopherol, as well as unsaturated fatty acids, chiefly arachidonic acid, in membrane phospholipids In view of the nature of the bond between α-tocopherol and phospholipids [9], it is assumed that conduction pores are formed at the peroxidation sites in the bilayers, and they cause the increase in membrane permeability.

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BIOPHYSICS

UDC: 576.331

QX-572, A QUATERNARY DERIVATIVE OF LIDOCAINE, USED TO BLOCK GATING CURRENTS IN THE MEMBRANE OF RANVIER'S NODE

Moscow DOKLADY AKADEMII NAUK SSSR in Russian Vol 244, No 6, 1979 pp 1492-1495

[Article by G. G. Gusel'nikova, E. M. Peganov and B. I. Khodorov, Institute of Surgery imeni A. V. Vishnevskiy, USSR Academy of Medical Sciences, Moscow (presented by Academician P. G. Kostyuk, 14 Oct 78), submitted 24 Oct 78]

[Text] In previous works conducted on isolated frog nodes of Ranvier, it was shown that the tertiary local anesthetic trimecaine [1] and neutral anesthesin [benzocaine] [2] have a blocking effect on sodium gating currents. A reversible decrease in gating charge was observed under the effect of novocain in experiments on the squid's giant axon [3].

Our objective here was to test the effect on sodium gating currents of compound QX-572, a charged quaternary derivative of lidocaine.

Experiments were conducted on isolated myelinated fibers of the frog's sciatic nerve. We recorded the potential by the method in [4]. We cut the internodal segments on either side of the tested node in isotonic KCl solution. When measuring ion currents, the node was perfused with normal Ringer's solution. Potassium currents were blocked by adding 10 mM tetraethyl ammonia chloride to the external solution. To measure shift currents, the sodium ion currents were depressed by changing the Na^+ in the external solution to $tris^+$ and adding $3\text{-}5\cdot10^{-7}$ M tetrodotoxin. Since compound QX-572, unlike most other quaternary analogues of local anesthetics, is quite soluble in lipids [5], we tested its blocking effect on sodium channels by means of external application to the node membrane. For all of the solutions, pH constituted 7.2; the temperature varied within the range of 12-15°C in the different experiments.

Gating currents were obtained as a result of algebraic summation of currents arising in response to switching square-wave voltage pulses on and off; these pulses were strictly identical in amplitude and duration, but of opposite signs. The total number of summations for each reading constituted 32. The minimal interval was 150 ms. An ATAS-250 digital averaging unit was used to average the current. Before averaging, the signal was cleaned by means of a passive filter with a gating band of up to 50 kHz. The data were displayed on an oscillograph screen. The following symbols are used here: E--potential of internal side of the membrane minus the external, taken as 0; V--shift of potential from base value (maintained by E_h); I_{Na}--sodium current; I_g--gating current; Q_{on}--charge shifted during response to switching on; Q_{max}--maximum value thereof; Q_{off}--charge shifted in response to switching off.

In all of the tests, QX-572 (0,2 mM) elicited a distinct decline in gating currents. Figure 1 illustrates an example of tracings of I_g obtained in response to test shifting of potentials V = 90 mV (a, c, e) and V = 100 mV (b, d, f) from E_h = -110 mV. In the top row (a, b) are shown control I_g's before treatment with QX-572. The bottom row (e, f) are tracings of I_g 5 min after addition of QX-572 to the perfusion liquid. We see that QX lowered the responses to turning the test pulses both on and off. There were no significant changes in kinetics. The middle tracings (c, d) show the same reversible changes in I_g as observed in the same experiment under the influence of depolarizing prepulses, which cause rapid inactivation of gating currents. These tracings were made prior to applying QX-572 to the node. The amplitude of the prepulses (V = 50 mV) and duration thereof (25 ms) were sufficient for virtually complete inactivation of I_{Na} in the control Ringer's solution. We were impressed by the great similarity of effects of prepulses and QX-572. This similarity becomes even more apparent if we examine the curves of the shifted charge Q_{on} as a function of test potential (Figure 2a). QX-572 and depolarizing prepulses induced about the same decline of Q_{on} with all test potentials. There was about 30% decline of Q_{max} ($\sim 1 \cdot 10^{-13}$ Kl [coulomb?]).

The bottom curve [3] corresponds to the difference between curves 1 and 2. It apparently characterizes the dependence on potential of the component of the shifted charge that was sensitivive both to QX-572 and depolarizing prepulses ("immobilized charge"). The same curves normalized to the maximum are illustrated in Figure 2b. A scrutiny of this Figure shows that QX-572 and inactivation elicited similar change in parameters of curve Q_{on}-E. The curve of the "nonimmobilized charge" (2) presents a lesser slope and its mean point is shifted to the left, in the direction of negative potentials, as compared to the curve of distribution of a full charge (1). Accordingly, the curve of the "immobilized charge" (3) presents a substantially greater slope and its mean point is shifted in the direction of higher V.

The obtained data can be interpreted as confirmation of the previously expounded hypothesis [1] that the pool of gating particles controlling permeability of the sodium channel is heterogeneous: some of these particles may be immobilized by the depolarizing prepulse or anesthetic, while others are resistant to both these factors. The difference in properties of these two types of particles is emphasized by the difference in parameters of distribution of the corresponding Q-E curves.

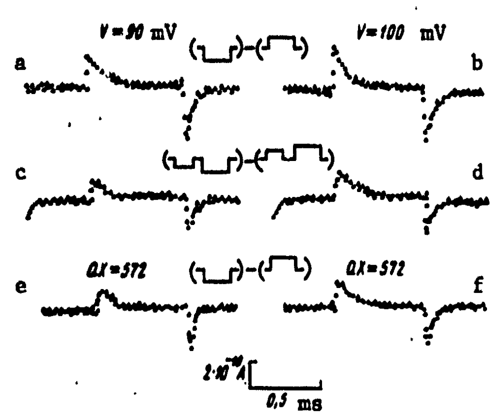
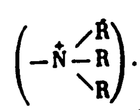


Figure 1. Effect of QX-572, quaternary derivative of lidocaine, on sodium gating currents in Ranvier node membrane compared to effect of rapid sodium inactivation. Symmetrical square-wave pulses were applied from a maintained potential of E = -100 mV. Tracings of gating currents with test pulse amplitude (V) of 90 and 100 mV.

a, b) in control sodium-free solution (Na^+ is replaced with $tris^+$) containing 300 nM tetrodotoxin and 10 mM tetraethyl ammonia chloride
c, d) effect of depolarizing prepulses (V = 50 mV lasting 25 ms)
e, f) effect of adding 0.2 mM QX-572 to control solution. Gating currents were recorded without prepulses

In the opinion of one of the present authors (E. M. Peganov), a basically different interpretation of these phenomena can be offered. It can be assumed that the pool of gating dipoles is homogeneous, but that there is a type of cooperative relations between them, with which immobilization of part of the charges leads to a change in interaction (cooperation) between the other particles. This leads to a change in parameters of curve Q-E [6].

According to conceptions prevailing at the present time, quaternary derivatives of local anesthetics are capable of interacting with a receptor situated in the internal orifice of the Na canal [7-10], binding with this receptor, QX blocks the canal with its cation head



The most important prerequisite for the block is opening of activation gates of the channel by depolarizing pulses [8]. The question of nature of chemical groups of this QX receptor is of great interest to identification of the molecular structure of the Na channel. The data obtained in this study concerning the inhibitory effect of QX-572 on gating currents, and the similarity of the effects of QX-572 and rapid inactivation warrant the assumption that ammonia compounds interact with the same gating particles that, according to the hypothesis in [11], are immobilized by depolarization of the inactivation subunit (h gates) of the Na channel. A recently published report [12] concerning partial decline of Q_{on} after injection of QX-314 (lipid-fast lidocaine derivative) in the squid's giant axon is quite consistent with the expounded hypothesis.

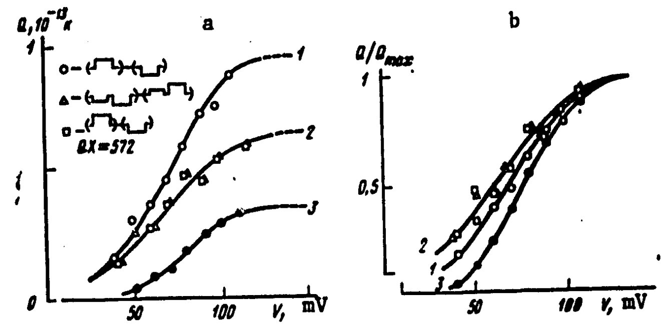


Figure 2. Stationary distribution of shifted charge

1) in control solution
2) responses to switching on after depolarizating prepulses, 25 ms, V = 50 mV (triangles) and effect of adding 0.2 mM QX-572 to control solution (squares)
3) difference between cuves 1 and 2
a) insert illustrates pulse program
b) the same curves as in a, but normalized to the maximum

The authors wish to express their appreciation to the Astra Firm, which kindly supplied compound QX-572 for the experiments.

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INDUSTRIAL MICROBIOLOGY

UDC 576.858:543

NEW METHODS OF PURIFYING VIRUSES FOR THE PREPARATION OF VACCINES

Purifying and Concentrating Viral Suspensions

Moscow VESTNIK AKADEMII NAUK SSSR in Russian No 1, 1979 pp 32-36

[Address by S. Ye. Bresler]

[Text] Opening the meeting of the Presidium concerned with new methods of purifying viruses for vaccine preparation, the president of the USSR Academy of Sciences, Academician A. P. Aleksandrov, spoke on the importance of results obtained and the nontriviality of the approach to the solution of this problem. Various aspects of the problem were discussed by S. Ye. Bresler (doctor of chemical science at the B. P. Konstantinov Institute of Nuclear Physics, USSR Academy of Sciences), E. A. Fridman (doctor of medical science at the Pasteur Institute of Epidemiology and Microbiology) and V. M. Kolikov (candidate of technical science at the M. I. Kalinin Leningrad Polytechnic Institute).

Preventative vaccination is a most effective method for fighting viral infections. But so that an administered dose of virus does not cause disease, viruses in a vaccine must be either changed from their native form or killed (as in the so-called inactivated vaccines). As is commonly known, viruses are cultured in large embryos or tissue cultures, so even for good virus yields in a final culture, viral proteins generally compose less than 1/1000 of the proteins of the embryo or tissue. When an organism is immunized the viruses must compete with a thousandfold excess of other antigens, i.e. inducers of immunity, and naturally the final effect obtained is weak. Moreover, the introduction of a great number of foreign proteins into an organism is fraught with many dangers: allergy, increased temperature and other sometimes serious complications are possible.

Obviously, in the preparation of a vaccine it is necessary to purify and concentrate viral suspensions. However, even now this is often not done. For example, contemporary rabies vaccine differs little from that which was used by Pasteur. Against tick-borne encephalitis a poorly purified, unconcentrated and consequently poorly effective vaccine is used. As for influenza vaccine, abroad inactivated vaccines are purified in ultracentrifuges. Since ultracentrifuges are expensive and short lived, this method is not economical and the cost of one dose of vaccine is very high. Moreover, as shown by American scientists in a recent experiment which was also confirmed in our laboratories, high pressures in ultracentrifuges (nearly 1000 atm) cause dissociation and loss of neuraminidase, one of the most important viral coat proteins. As a result the immunological properties of the purified virus are altered and do not correspond to the properties of virus circulating through the population.

Workers of the B. P. Konstantinov Leningrad Institute of Nuclear Physics, USSR Academy of Sciences, the M. I. Kalinin Leningrad Polytechnic Institute and the Pasteur Institute of Epidemiology and Microbiology developed a new method for purifying and concentrating viruses using adsorption chromatography.

For separating molecules and ions the chromatographic method was shown to be exceptionally effective and highly resolving. But its application to viruses, i.e. complex structures consisting of thousands of protein, nucleic acid and lipid molecules, was completely unstudied. To us, questions on the reversibility of adsorption-desorption and on the stability of virions (virus corpuscles) became cardinal. Since adsorption-desorption is repeated many times in the chromatographic process, the requirement of reversibility is very strict. An experiment showed that adsorption and chromatography of viruses on a rationally selected adsorbent proceeds reversibly and that the virions are not damaged in this process. Thus the proposed method was approbated. Based on this a technological method for vaccine preparation was developed. The originality of the method is attested to by the fact that patents have already been given for it in certain countries.

The choice of adsorbent is very important in solving a problem by chromatographic purification. The dimensions of its pores must allow virions to penetrate into the pores. The diameter of an influenza virus is 1100 angstroms.

A suitable adsorbent, macroporous glass, was first developed by S. P. Zhdanov with coworkers in the Institute of Silicate Chemistry, USSR Academy of Sciences. They determined that by fusing silicic and boric anhydrides and annealing the mixture it is possible to obtain a system of the finest droplets. After grinding, the boric acid is removed from them by the action of base and acid. This method was first mastered by the Gor'kovskiy Experimental Plant. For the first time in the country the production of an adsorbent--macroporous glass with pore dimensions of about .5 micrometers--was set up for use in the production of influenza vaccine.

The chromatographic purification of viruses was studied in depth with respect to physical chemistry. It was determined that virus adsorption is reversible and depends strongly on the concentration of hydrogen ions in the medium. In order to quantitatively characterize the affinity of viruses for the glass surface, the isothermal adsorption was measured, i.e. the dependence of adsorption on the virus concentration in the aqueous suspension. In working with such an unusual substance as viruses, special methods had to be applied for measuring their concentration. The number of virions per unit volume was counted directly with an electron microscope. It was possible to carry out such an experiment, since we possessed a method for obtaining viruses in highly purified form.

Saturation of the adsorbent's surface with influenza virus takes place at the very low concentration of 10^{-11}M (the concepts of gram-molecule and molar concentration apply to virus corpuscles), i.e, the process occurs with a large decrease in free energy (on the order of 20 kcal/mol). Adsorption increases as the temperature rises (figure 1). Consequently, it is not caused by a reduction of energy but by an increase in entropy. This is highly characteristic of hydrophobic interactions--when adsorbed particles have water repelling properties. Since influenza virus, like most other viruses, is covered with a film of lipids, such adhesion to glass is quite natural.

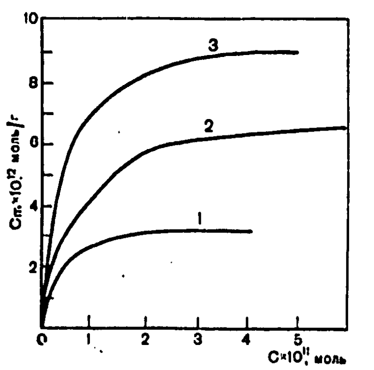


Figure 1. The isothermic adsorption of influenza virus A_2 on macroporous glass at various temperatures.

1. 15 C, 2. 24 C, 3. 35 C
C is the molar concentration of viruses in solution. C_m is the amount of viruses adsorbed in grammoles per gram of glass.

All that has been said applies to electrically uncharged glass, i.e., glass in a neutral medium. But the surface of glass is covered with silanol groups (SiOH), which are negatively charged in a weakly basic reaction environment. Negatively charged virions are repelled from the charged glass, and under critical conditions (when repulsion is equalized by adhesion) a whole layer of viruses is washed from the glass (figure 2). The process occurs so abruptly that we call the phenomenon critical adsorption-desorption.

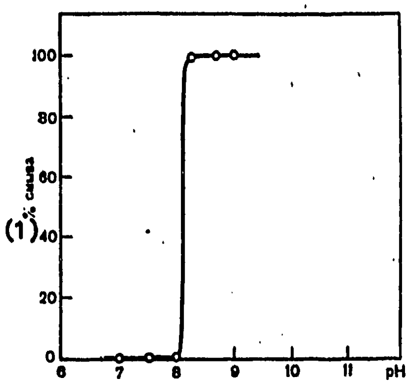


Figure 2. The dependence of the desorption of A_2 influenza viruses on hydrogen ion concentration.

Key:

1. Percent eluted

The physical reason for the abruptness of the phenomenon is clear. The free energy of binding, as already mentioned, is approximately 20 kcal/mol. Ther thermal energy of oscillation for the virion relative to the surface is 600 cal/mol, which is only 3 per cent of the binding energy. Therefore, the viruses can be eluted in a small volume with a 20-30 times increase in concentration.

An understanding of the role of hydrophobic interactions allowed a subsequent increase of desorption by adding organic solvents. The critical conditions for desorption differ sharply not only among different viruses but even in different strains of the same virus (figure 3). This creates favorable conditions for the chromatographic process--the release of viruses is total and sharp. This allows not only purification from all admixtures but also concentration of the viruses.

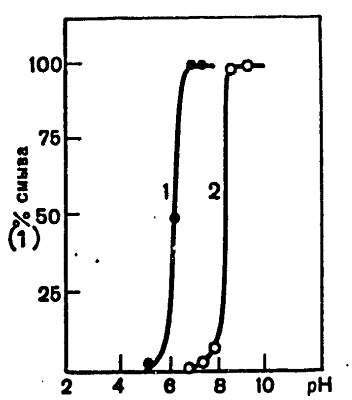


Figure 3. The dependence of the desorption of A_0 and A_2 influenza viruses on hydrogen ion concentration. 1 - A_0, 2 - A_2

Based on the physicochemical study of virus absorption chromatography a technologically acceptable method was developed for preparing vaccines against influenza, thick-borne encephalitis, poliomyelitis and rabies. Influenza vaccine is already produced on a semi-industrial scale and is undergoing massive testing. Therefore the remainder of this speech will refer to it, although the method of purification and concentration has been shown effective for all tested viruses.

The developed method combines several procedures which give potential for adjusting the method to new influenza virus strains. Production begins with reproduction of an influenza virus which the World Health Organization reports as having a dangerous distribution in early fall.

The virus is cultured in ten-day old embroys. Two days after an embryo is infected the allantoic fluid is drawn off under vacuum. Each milliliter of this fluid contains over 10^9 virions. The liquid is developed on a chromatographic column filled with large-pored glass (pore diameter 5000 angstroms). The column is saturated with viruses which are later eluted in a small volume at pH 8.5. About 100 l of allantoic fluid is required to saturate the column, and a volume of 3-4 l is used for elution. In all, the viruses are purified approximately 1000-fold and concentrated about 30-fold (figure 4).

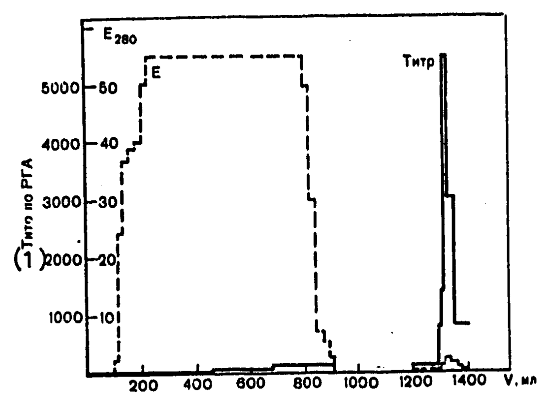


Figure 4. Chromatograms of A_2 influenza viruses on a macroporous glass column

On the right are curves of desorption from the column; curves on the left characterize column absorption; the dotted curve represents the totalamount of protein as measured by light absorption at 280 nm; the continuous curve shows the titer of viruses.

Key:
1. Titer by hemagglutination inhibition

In the laboratory of academician G.N. Flerov (Joint institute of Nuclear Research) a method was developed for producing membranes necessary for this purpose. Holes are made in lavsan film by ionic bombardment in a cyclotron and subsequent etching in base. Unlike cellulose membranes, nuclear filters have strictly calibrated holes which greatly facilitate the solution of our problem.

For the concentration of several virus strains, the adsorption-desorption method was applied to the surface of erythrocytes. Sometimes it was possible to additionally purify an already concentrated virus suspension by gel filtration on the same macroporous glass. The glass surface is chemically treated and as a result becomes extremely hydrophobic, i.e. absolutely unadsorbing. A viral suspension is passed through a small column filled with the modified glass. The glass pores are selected so that impurities penetrate into them but virions do not. When a suspension is passed through the column, viruses pass more rapidly and impurities remain behind. This process is called gel filtration. It is not always necessary to apply the whole arsenal of methods; the combination selected depends on the virus.

The final process is inactivation of the viruses with ultraviolet light of 254 nm wavelength. Inactivation proceeds with a strict dose in specially constructed devices. After passing all of the necessary control steps, the virus is ready for issue.

The scale of vaccine production by a semi-industrial plant in Leningrad was 200,000 doses per month in the autumn of 1977. On the average 3 doses are obtained from one embryo. The cost of a dose is about 60 kopecks. During September-November 1977 over 600,000 doses were issued and used for vaccination of the Leningrad population.

During state tests conducted by the L.A. Tarasevich State Institute for Standards and Control of Medicinal Biological Preparations, the vaccine was shown to be highly effective. The sick rate for people who had been vaccinated was 3.5 times less than for those unvaccinated.

The technology of virus purification by chromatography can be realized on an industrial scale--for the production of ten million doses. In addition it is necessary to extend it to other viruses, since the described method applies also to their purification and concentration.

Administration of Inactivated Vaccines

Moscow VESTNIK AKADEMII NAUK SSSR in Russian No 1, 1979 pp 36-39

[Address by E.A. Fridman]

[Text] Inactivated influenza vaccine is a suspension of virions. It belongs to the category of corpuscular vaccines, where the virions are not damaged and maximally purified. It suffices to say that the protein content of vaccine issued in 1977 does not exceed 100 micrograms/ml (not more than 20 micrograms/ml in a dose). This is a minimal protein content for vaccines in this category. The concentration of virions is approximately 10^{11} per milliliter. According to our observations such a concentration gives satisfactory immunity, i.e. ability of the preparation to cause the formation of protective antibodies in an organism.

After each dose of inactivated vaccine is administered the content of influenza antibodies in the blood of a human sharply increases (figure 1, table 1). It is commonly known that a titer of 80 hemagglutination units of antibodies per .2 ml blood serum is sufficient to protect an organism from homologous influenza virus strains. All the values in table 1 significantly exceed this level.

Table 1. The immunological activity of inactivated chromatographed monovaccines obtained from various influenza virus strains

Вакцинный штамм (1)	Число привитых (2)	Частота * сероконверсии, % (3)	Средний геометрический титр антител (4)		Средняя кратность сероконверсий (7)
			в I сыворотке (5)	во II сыворотке (6)	
(8) A (Гонконг) 68	20	95	19,7	338	17,1
A (Виктория) 72 (9)	31	83	16	315	19,7
(10) A (MRC) 11	30	80	8,7	218	25
A (Шотландия) 74 (11)	42	86,6	34	239	7
(12) A (Ленинград) 76	21	80,9	18,4	147	7,4
»	80	78,7	17,1	147	8,8
(13) B (Япония)	18	72,2	34	294	8,6

*in persons with original antibody titer 1:20

Key:

1. Vaccine type
2. Number innoculated
3. Frequency of serum conversion
4. Geometric mean of antibody titers
5. In I serum
6. In II serum
7. Mean multiplicity factor for serum conversion
8. A (Hong Kong) 68
9. A (Victoria) 72
10. A (MRS) 11
11. A (Scotland) 74
12. A (Leningrad) 76
13. B (Japan)

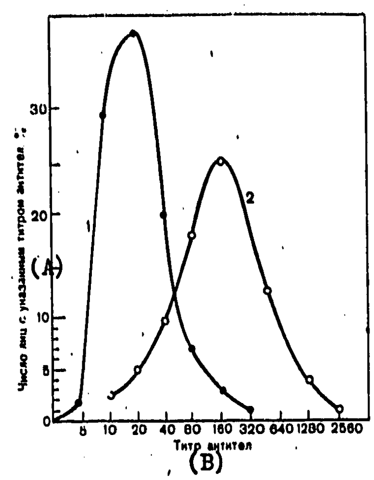


Figure 1. Distribution of indicators of humoral immunity. Vaccine A (Victoria) 72 was administered once (0.5 ml subcutaneously).
1-the sick rate of unvaccinated persons
2-the sick rate of vaccinated persons

Key:
- A. Number of persons with the indicated antibody titer (per cent)
- B. Antibody titer

An experimental series of vaccines was tested against various virus strains. It was determined that this method is universal; it is possible to prepare vaccine against any strain which has appeared (see table 1).

Different doses of vaccine were tested. A final decision on the optimal dose will be passed by the Institute of Standards and Control of Medicinal Biological Preparations. However, it is already now possible to say that the dose is not large; .2 ml vaccine provides brilliant results (table 2).

Table 2. Results of a blood serum study of persons innoculated with inactivated influenza vaccine during fall 1977 at Leningrad industrial plants

Коллектив (1)	Доза вакцины, мл (2)	Число обследованных лиц (3)	(4) Из них с достоверным нарастанием титра антител: (5) абс.	%	(6) Средний геометрический титр антител: (7) до прививки	(8) после прививки	Кратность нарастания титров антител (9)
№ 1	0,1	199	145	72,8	14,9	158	10,6
№ 2	0,2	112	102	91	15	316	21

Note—the vaccine was administered by intracutaneous injection.

Key:
1. Group
2. Vaccine dose (ml)
3. Number of examined persons
4. Those with sufficient accumulation of antibodies
5. Absolute number
6. Geometric mean of antibody titers
7. Before vaccination
8. After vaccination
9. Multiplicity factor for antibody titers

A very important property of the preparation is its safety. A weak reaction is very seldomly observed when the vaccine is directly administered. Attempts were made to determine whether or not administration of the vaccine reduces resistance to other diseases. At the left of table 3 is a listing of diseases which appeared in people during seven months after innoculation; influenza and other acute respiratory diseases are not the only diseases included. It was determined that the innoculated and uninnoculated per-centages of those did not depend on whether the preparation was administered or not. Consequently, the vaccine can be concluded safe.

Table 3. The total sickness among persons vaccinated with inactivated chromatographed vaccine during the seven months after vaccination (excluding influenza and other acute respiratory diseases)

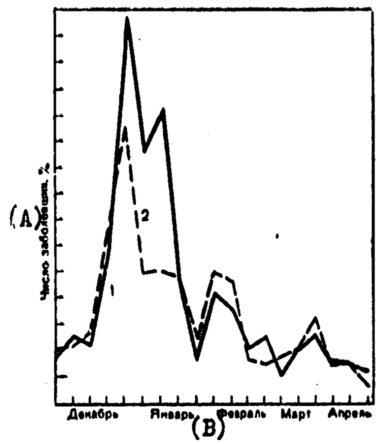
Перечень болезней (1)	(2) Число заболеваний	
	привитые (3) (1419 чел.)	непривитые (611 чел.) (4)
(5) Ангина, обострение хронического тонзиллита, бронхиты, пневмонии, туберкулез, острые желудочно-кишечные заболевания, хронические болезни желудка, болезни печени и почек, невралгии, невриты и прочие болезни нервной системы, ревматизм, аллергические заболевания, гнойные процессы, болезни кожи	257 (18,11%)	135 (22%)

Key:

1. List of diseases
2. Number of illnesses
3. Vaccinated (1419 persons)
4. Unvaccinated (611 persons)
5. Angina, aggravated chronic tonsillitis, bronchitis, pneumonia, tuberculosis, acute gastric-intestinal disease, chronic stomach sickness, liver and kidney diseases, neuralgia, neuritis and nervous system disorders, rheumatism, allergic diseases, suppurative processes, skin disease

Data from state experiments conducted in 1974-1975 attests to the high epidemiological effectiveness of inactivated vaccine (figure 2). In one case the preparation was administered at the height of the epidemic. The well timed administration of vaccine gives even more convincing results (figure 3).

There is still another possible use of vaccination--for the preparation of anti-influenza donor medicinal gama-globulin. This method has been known and applied since 1964. It is an extraction of blood serum containing great quantities of influenza antibodies. Since the preparation is made from donor blood, its production is limited and suitable primarily in pediatrics.



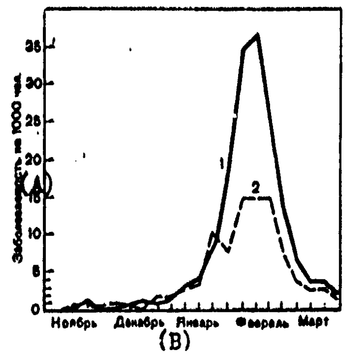


Figure 2. The incidence of influenza and acute respiratory infections among a group observed in 1974-1975 (Data from (GISK) 1--sickness among those given a placebo (saline solution not containing vaccine, 2--sickness among those vaccinated with A(Victoria) 72 vaccine

Key:
- A. Number sick (per cent)
- B. December, January, February March, April

Figure 3. The incidence of influenza in the Poligrafmash plant during 1975-1976. Vaccine A(Scotland) 74

1--sickness of the unvaccinated
2--sickness of the vaccinated

Key:
- A. Sickness per 1000 people
- B. November, December, January, February, March

Vaccination of donors with inactivated vaccine increases the content of influenza antibodies in their blood 6-12 times more than does administration of other vaccines. Thus the activity of the preparation sharply increases. The Pasteur Institute of Epidemiology and Microbiology took on the obligation to provide this type of vaccine to all plants in the country which produce this gama-globulin. Donors are vaccinated once per year. Observations are also made on the safety of repeated vaccination.

Up to recent times vaccination was quite expensive. Now the cost of preparation has been reduced to 90 kopecks per dose. A real possibility exists for lowering it further.

New Method for Virus Purification

Moscow VESTNIK AKADEMII NAUK SSSR in Russian No 1, 1979 pp 39-42

[Address of V.M. Kolokov]

[Text] The basis of a new method for virus purification is the phenomenon of critical adsorption-desorption.

The problem in a practical scheme is to realize this phenomenon and thereby obtain the necessary degree of virus concentration and purification.

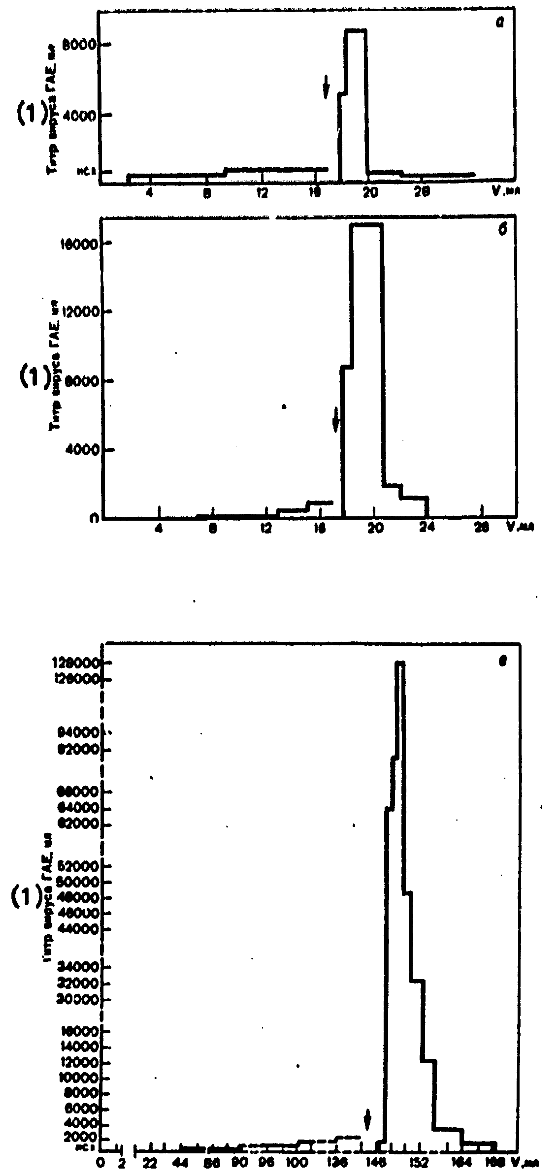
The complexity of this problem is determined by the properties of the viral suspension. Components of the suspension not only hinder virus adsorption but also make it frequently irreversible. In order to develop the effect the solution is subjected to preliminary purification (the substances interfering with virus adsorption are removed) and an optimal adsorbent possessing high adsorption capacity is selected.

The adsorption capacity is determined by the amount of surface accessible for virus adsorption, and this to a large extent depends on the pore dimensions. It increases with pore size, since the larger the pores, the more surface available for virus adsorption. An increase in the capacity leads to an increase in the virus concentration and purity after desorption. This tendancy is demonstrated by a series of influenza virus chromatograms.

In the eluate from a column of macroporous glass with pore radii of about 700 angstroms the virus concentration reaches several thousand hemagglutination units. The degree of concentration is 10-15 times. When glass with 3000 angstrom pore radii is used we obtain a greater degree of concentration (20-30 times) and ten thousand hemagglutination units per milliliter. In the eluant from a glass column with pore radii of about 8000 angstroms the concentration is increased a hundredfold (see figure 1a,b,c) and reaches a hundred thousand hemagglutination units. Such a concentration is obtainable only with an ultracentrifuge. A simple column filled with glass is becoming competitive with a most complex machine—the ultracentifuge.

Close collaboration with the I.V. Grebenshchikov Institute of Silicate Chemistry, USSR Academy of Sciences, and particularly with the laboratory of S.P. Zhdanov allowed us to obtain and test new, more ideal forms of macroporous glass.

All that has been said shows the possibilities of the adsorbent and the method. Now the task is to work out the technology, i.e. the realization of critical desorption on an industrial scale.



Chromatograms of influenza virus on a column with macroporous glass gaving various pore radii: A--radius is approximately 700 angstroms, B--3000 angstroms, C--8000 angstroms

Key:

1. Virus titer (Hemagglutination units per milliliter)

A.Y. Dudorov (director of the Institute for Work Hygiene and Professional Disease) reported that approximately five hundred thousand residents of Leningrad were vaccinated in 1977. Not being able to speak on the final effectiveness of the immunization, he listed several undoubted merits of the new vaccine. First, it does not cause any unfavorable responses in humans. Secondly, the method of administration is so simple that many people can be innoculated in a short time, and when a high percent of the population is vaccinated a collective immunity is created. Finally, instracuteneous administration of the vaccine guarantees immunization.

K.P. Seleznev (Leningrad Polytechnic Institute) stressed that the creation of the new method for virus purification is the result of cooperation between physical chemists and engineers. All of the experimental apparatus was developed and manufactured in the Polytechnic Institute. The faculty of the biophysics institute are developing several important scientific areas and they are also training engineer-biophysicists. However, more than once questions have been raised on the enclosure of the faculty as not conforming to the profile of the institute. K.P. Seleznov asked the Presidium of the USSR Academy of Sciences to support the faculty's lines of work.

Corresponding member of the USSR Academy of Sciences M.M. Shul'ts devoted his address to the problem of organizing the production of porous glasses. The production of glass with regular porosity is of fundamental importance for the creation of various vaccines. In the Institute of Silicate Chemistry, USSR Academy of Sciences, the laboratory of S.P. Zhdanov is successfully working in this direction, but in order to set up extensive vaccine production it is necessary to organize the production of porous glass on an industrial scale.

A.I. Rublevskiy (Joint Institute of Nuclear Physics) drew attention to the organizational complexities in the production of nuclear filters, which are used for additional vaccine purification. It is extremely important to organize their production on a massive scale.

A.N. Rublevskiy was supported by academician M.A. Markov. He stressed that nuclear filters can be used not only for purifying viruses but also for other most diverse purposes. In the opinion of M.A. Markov, it is essential that state bodies organize the production and broad introduction of the new filters into national industry.

T.V. Peradze (director of the Pasteur Institute of Microbiology and Epidemiology) spoke on yet another extremely important feature of the new method for vaccine production--the speed of the process. While the preparation of live influenza vaccine requires over ten months, the new method permits thepreparation of vaccine in only one and a half months (including all control steps). Thus, one and a half months after a new virus appears in the country, a vaccine can be prepared and used in practical public health service. The vaccine is also very effective for the production of active and highly purified donor gama-globulin. Whereas 5-6 innoculations were previously required to produce a sufficient quantity of antibodies in the blood of donors, now one vaccination per year is sufficient. T.V. Peradze noted that success in creating the new method for purifying vaccines was provided by the collaboration of physicists, doctors and engineers.

The vice president of the USSR Academy of Sciences, Y.A. Ovchinikov suggested that the wave of viral disease is growing. Inactivated vaccine is a very active agent, and the technology of its preparation is original and progressive. The actual problem is to organize the production of vaccine in industrial quantities and to apply it in general. Y.A. Ovchinkov urges that the Presidium turn to the USSR Ministry of Public Health with a proposal to speed introduction of the new method for preparing vaccines into industry and to draw other services into work on this task. The vice president also underscored that the methods connected with chromatography on porous glass and universal and are applied in many fields of science and industry. It is necessary to in every way promote work in this area.

In conclusion, the president of the USSR Academy of Sciences, academician A.P. Aleksandrov said that active support was contributed to the development of the new method for virus purification by both the Academy of Sciences and the Leningrad party organization. This helped Leningrad scientists to rather rapidly obtain important results. The time between the onset of an epidemic and the first knowledge of the disease is about three months. The new method, which requires only one and a half months for the preparation of vaccine for an identified virus strain, permits operational preparedness for an epidemic. Academician A.P. Aleksandrov expressed the opinion that the work of Leningrad specialists deserves great appreciation and is worthy to be nominated for prize competition.

Resolution of the USSR Academy of Sciences

Moscow VESTNIK AKADEMII NAUK SSSR in Russian No 1, 1979 pp 42-43

[Text] The Presidium of the USSR Academy of Sciences accepted a resolution approving the work of the B.P. Konstantinov Institute of Nuclear Physics, USSR Academy of Sciences, the M.I. Kalinin Leningrad Polytechnic Institute, and the Pasteur Institute of Epidemiology and Microbiology on creating new methods of purifying viruses for vaccine preparation and for the production of inactivated influenza vaccine.

A decision was made to request the RSFSR Ministry of Higher and Secondary Education and the RSFSR Ministry of Public Health to commission the M.I. Kalinin Leningrad Polytechnic Institute and the Pasteur Institute of Epidemiology and Microbiology togehter with the B.P. Konstantinov Leningrad Institute of Nuclear Physics to finish working out technology for the production of influenza vaccine. These institutions are to estimate the possible industrial undertakings on which this technology will be applied and to accelerate development of the production of vaccines for tick-borne encephalitis and rabies.

The Presidium of the USSR considers indispensable the organization of the production of DV-1 glass with primary thermal treatment according to the method proposed by the I.V. Grebenshchikov Institute of Silicate Chemistry, USSR Academy of Sciences. This glass is to be provided to the Gor'kovskiy Experimental Plant of the All-Union Science Research Institute of the Petroleum Refinery Industry.

The production of macroporous glass from DV-1 glass should be organized at the Gor'kovskiy Experimental Plant in accordance with technology developed by the I.B. Grebenshchikov Institute of Silicate Chemistry, USSR Academy of Sciences, together with the plant.

The Presidium of the USSR Academy of Sciences decided to request the USSR State Committee on Science and Technology and the RSFSR Council of Ministers on the Use of Atomic Energy to secure work on the preparation of nuclear filters in the laboratory of Nuclear Reactions, Joint institute of Nuclear Research, having given out assignments, state units, materials and equipment necessary for these goals.

It is resolved to ask the RSFSR Ministry of Higher and Secondary Special Education and the USSR State Planning Commission to strengthen the material base of the biophysics department at the Leningrad Polytechnic Institute, RSFSR Ministry of Higher Educational Institutions, and to organize the training of young specialists in the specialty Engineering Biophysics. It is also resolved to request the USSR State Committee on Science and Technology to speed the organization of a laboratory in the Leningrad Polytechnic Institute for the technology of antivirus preparations.

The Presidium of the USSR Academy of Sciences decided to request the RSFSR Ministry of Public Health and the RSFSR Ministry of Higher and Secondary Special Education to award prizes to workers of the Institute of Epidemiology and Microbiology and the Leningrad Polytechnic Institute who have taken part in the creation of inactivated influenza vaccine. The Presidium also recommends that awards be given to workers who have participated in the application of the new vaccine to vaccination of the Leningrad population.

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MICROBIOLOGY

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TOXIGENICITY AND ENTOMOPATHOGENICITY OF DIFFERENT BACTERIA STRAINS OF THE BACILLUS CEREUS-THURINGIENSIS GROUP

Moscow DOKLADY VSESOYUZNOY ORDENA LENINA AKADEMII SEL'SKOKHOZYAYSTVENNYKH NAUK IMENI V. I. LENINA in Russian No 2, 1979 pp 17-18

[Article by Dr Sci O. Lysenko, Dr Biol Sci N. V. Kandybin, and A. A. Stus', Czechoslovak Academy of Sciences Institute of Entomology, and the All-Union Scientific Research Institute of Agricultural Microbiology]

[Text] The toxigenicity of bacteria is often utilized in infectious entomopathology for their identification. One of the principal diagnostic characteristics of *Bacillus thuringiensis* is the crystalline entomocidal endotoxin they form. But under certain conditions on one hand these bacilli lose their ability to produce crystalline endotoxin and, on the other hand, they easily recover it on entering the body of a susceptible insect (5). We know that some microorganisms produce a large number of metabolites having entirely different degrees of toxicity to insects. It would be theoretically valid to assume that the virulence of a microbe depends in the end on the cumulative action of all of the pathogen's toxic metabolites on its host (3,4).

Scientists of the All-Union Scientific Research Institute of Agricultural Microbiology are conducting a detailed study of this problem jointly with the Institute of Entomology of the Czechoslovak Academy of Sciences on the basis of a bilateral CEMA agreement.

The goal of our joint research is to study the correlative dependence between the entomopathogenicity of *Bac. thuringiensis* and *Bac. cereus* and their enzymatic and toxigenic activity. We used 10 strains of *Bac. thuringiensis* and five strains of *Bac. cereus* (from the collection of the insect pathology laboratory of the Czechoslovak Academy of Sciences Institute of Entomology). For some of them we first determined the extent of production of enzymes and entomocidal toxins--proteases, phospholipases, "egg yolk clearing" factor, thermostable exotoxin, and crystalline endotoxin.

The entomopathogenicity of the bacterial strains was studied with a laboratory population of the bee and flower moths (*G. mellonella, Ephestia* *kuhniella*), and with natural populations of the brown-tailed moth (*Euproctis chrysorrhoae*), the lackey moth (*Malacosoma neustria*), the satin moth (*Stilpnotia salicis*), and the fruit-tree ermine moth (*Hyponomeuta podellus*).

The bacterial strains were grown in 750 ml test tubes containing 35 ml medium (corn extract--3 percent, glucose--0.5 percent); the test tubes were placed in an agitator (180 rpm) at 28° for 72 hours.

The insects were infected perorally or by injection of the pathogen into the body cavity.

For peroral infection, feed treated with microbial suspension having a spore titer of 25, 50, 100, and 200 million/ml was fed to caterpillars in age classes I and II. Each dose was used on 10-20 caterpillars with threefold replication.

For parenteral infection, sterile culture fluid filtrate or its 1:10 and 1:100 dilutions were injected into the body cavity of *Galleria mellonella* age class VI caterpillars at a dose of 0.006 ml per specimen. The filtrate was obtained through a Zeiss bacterial filter. Insect death was determined after 3, 5, 10, and 15 days. To assess the virulence of individual strains, we felt it suitable to compute the DL_{50} for each strain by the graphical sampling method. Analyzing the data we considered only those values which yielded to computation within the given range of doses, the DL_{99} of which did not go beyond this range.

All strains were subdivided into three groups in relation to their virulence with respect to the listed insects. The first, most virulent group contained four strains of *Bac. thuringiensis*, two of which were of the first serotype (No 898 and No 058), one was of the third (No 751), and one was of the sixth (No 902). All of these strains had a pathogenic action on experimental insects in relatively small doses. The second, less virulent group contained seven strains, of which five were *Bac. thuringiensis* and two were *Bac. cereus* (No 905 and No 906). The virulence of this group was significantly different from that of the first. The third, least virulent group contained four strains, to include one strain of *Bac. thuringiensis* var *finitimus* and three strains of *Bac. cereus*. The entomopathogenicity of strains in the third group turned out to be very low, while the enzymatic activity of this group was not inferior to that of strains in the first and second group, and in relation to production of proteases, phospholipases, and hemolysins one strain of *Bac. cereus* was significantly superior to all of the other 14 strains.

Evaluation of the strains in relation to their enzymatic activity, toxigenicity, and virulence to insects would demonstrate absence of a correlation between these indices.

Thus all strains of the first, most virulent group form crystalline toxin. The latter is also formed by four strains of the second, less virulent group, in which case strain No 902 of the first group and strain No 903 of the second group are also similar in antigenic structure. Strain No 904 of the second group produces not only crystalline toxins but also thermostable exotoxin, and in relation to virulence it is inferior to strains of the first group, some of which do not form exotoxin.

Consequently the amount of toxic metabolites formed by the strains *in vitro* and their N-antigenic structure are not sufficient to describe the entomopathogenic characteristics of the strain *in vivo*. There can be no doubt that such a generalization contains a number of limitations stemming from the choice of the material for study and the methods employed. It is probable that given more-thorough multivariational analysis of all of the experimental material, some other dependencies could have been revealed. Moreover the accuracy of DL_{50} values obtained by means of per os infection are relative. On the other hand infection through feed is closer to natural conditions than forced-feeding. The advantage and the relative dependability of our results lie in the multifactorial nature of our experimental material, which was obtained with many bacterial strains and several insect species. Hence the obtained values are mutually comparable, and their relative dependability is satisfactory.

From a theoretical standpoint the rejection of a dependence between toxin formation and virulence sounds paradoxical. But such cases are not infrequent, and they require explanation. Similar paradoxes were revealed in research on the mutual relationships between entomopathogenic bacteria, nematodes, and insect hosts (4). Bacterial strains exhibiting the highest virulence to insects lost this property when in association with parasitic nematodes, while weakly virulent strains, on the other hand, caused extensive death of insects when in such an association.

Different strains of *Bac. cereus*, *Bac. pumilus*, and *Bac. licheniformis* differed noticeably among one another in relation to entomopathogenicity: Some caused 100 percent mortality of bee moth caterpillars injected with 40,000 cells, while others had no effect when injected at a dose of 250,000-1,000,000 cells (1). In our opinion such anomalies can be explained mainly by the instability of action of toxic metabolites, which depends on the conditions and place of manifestation of this action, not to mention the susceptibility of the macroorganism and its protective mechanisms. Moreover, we know that many microbial metabolites become toxic only when combined with other nontoxic metabolites.

Consequently the entomopathogenicity of a microbe cannot be reduced to just the action of its toxic metabolites alone.

Many important problems in the pathogenesis of insect bacterial infections have not been studied yet, which is even true for infection elicited by *Bac. thuringiensis*. Deeper joint research on microbial entomopathogenicity under CEMA sponsorship will doubtlessly promote further development of microbiological science in general and infectious insect pathology in particular.

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VETERINARY MEDICINE

UDC 636.2:616.155.392

IMMUNOLOGICAL PROPERTIES OF TYPE *C* CATTLE ONCORNAVIRUS

Moscow DOKLADY VSESOYUZNOY ORDENA LENINA AKADEMII SEL'SKOKHOZYAYSTVENNYKH NAUK IMENI V. I. LENINA in Russian No 2, 1979 pp 35-36

[Article by Cand Biol Sci Kh. S. Salimov, Uzbek SSR Order of the Red Labor Banner Scientific Research Veterinary Institute]

[Text] One of the main tasks in research on cattle leukosis is timely diagnosis of disease and implementation of the appropriate control measures. Diagnosis of leukosis in the living animal on the basis of clinical hematological analyses is very laborious, and it is unable to reveal all sick cattle suffering and a leukemic disease course.

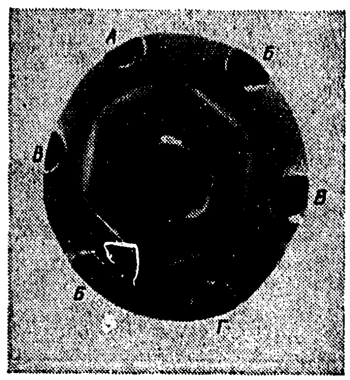
As a consequence there is much interest in developing immunological diagnostic methods. Immunological analyses of type *C* oncornavirus isolated from primary cultures of leukocytes from cattle stricken with leukosis have been acquiring special significance in recent years (1-8). According to a number of authors (8,9) type *C* oncornaviruses appear first in the leukocytes in organs of animal stricken with leukosis, while precipitating antibodies to these viruses are detected in blood serum; hematological and histological changes are detected subsequently. Considering this, research on precipitating antibodies to oncornavirus in animal blood serum has great significance to leukosis control measures.

The research was conducted jointly with colleagues of the Moscow Veterinary Academy's Problematic Laboratory of Leukosis Research and the USSR Academy of Medical Sciences' Institute of Virology imeni D. I. Ivanovskiy.

Oncornavirus isolated from primary leukocyte cultures and lymph node cells from cows stricken with leukosis was used as the antigen.

The (Oukhterloni) precipitation reaction in agar gel was employed. We used 1.2-1.5 percent Difko agar in physiological solution, pH 7.2 in the reaction. Craters in the agar were seeded with standard strains using seven tubular punches with an outer diameter of 4 mm, the distance between the outside and central tubes being 5 mm. The reaction was allowed to proceed at room temperature in a chamber humidified with 0.8-1.5 percent carbolic acid. The reaction was observed after 24-48 hours of incubation, and the results were recorded photographically. The precipitation bands were intensified with 0.065 percent cadmium sulfate solution after rinsing the sample with 0.15 M sodium chloride solution.

Blood serum from five groups of animals was studied to reveal precipitating antibodies to the purified oncornavirus.



Diffusional Precipitation Reaction: Center--oncornavirus antigen, peripheral craters--blood serum: *A*--From cow No 101, stricken with leukosis; *B*--from cow No 355, stricken with leukosis; *C*--control--antioncornavirus serum; *D*--From experimentally infected calf No 681.

Precipitating antibodies to type *C* oncornavirus were revealed by the immunodiffusion reaction in blood serum from two cows infected with cellular suspension when 1-10 days old, and in blood from a cow showing clinical signs of leukosis.

A precipitation band was discovered in the immunodiffusion reaction for blood serum from 7 out of 18 calves (38.8 percent) infected with purified oncornavirus, indicating presence of precipitating antibodies in the blood serum. It should be noted that type *C* oncornavirus having all of the physicochemical properties inherent to it was detected and isolated in leukocyte cultures from two calves and in lymph node cells from two calves 6-12 months following infection. The blood indices of these animals are within the physiological norm.

Precipitation antibodies to cattle oncornavirus were not revealed in blood serum from four calves infected with *Mycoplasma*.

Precipitating antibodies were detected in blood serum from 31 out of 42 (73.8 percent) cows testing hematologically positive for leukosis (the leukocyte count averaged 31,700±1,580, the percentage of lymphoid cells per milliliter of blood was 88.88±3.00 percent).

Precipitating antibodies were revealed by the immunodiffusion reaction in the blood serum of only 6 out of 98 healthy cows (6.12 percent).

Thus we established oncornavirus infection in 38.8 percent of the calves 6-12 months after infection, in 73.8 percent of the cows stricken with leukosis, and in 6.12 percent of hematologically healthy animals.

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11004
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VETERINARY MEDICINE

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DUCKLING RESPIRATORY DISEASE CAUSED BY INFLUENZA VIRUS

Moscow DOKLADY VSESOYUZNOY ORDENA LENINA AKADEMII SEL'SKOKHOZYAYSTVENNYKH NAUK IMENI V. I. LENINA in Russian No 2, 1979 pp 36-37

[Article by Cand Biol Sci U. S. Saidov, Republic Scientific-Production Veterinary Laboratory of Avian Diseases]

[Text] An outbreak of disease occurred among ducklings of one of the duck farms of Tashkentskaya Oblast in October 1977, exhibiting signs of affliction of the upper respiratory tract. The disease was observed in young birds from 5 to 30 days old. Disease was not noted among birds older than 30 days. The disease struck all broods, causing mass bird mortality (up to 64 percent).

The disease proceeded in its acute form, and in most cases the sick birds died. It began with the following clinical signs--sneezing and serous discharges from the nose, the discharge becoming purulent later. Breathing was encumbered and shallow. The ducklings breathed through open beaks since the external nares were clogged with fibrinous-caseous matter. Conjunctivitis was observed. The mucous-purulent exudate underwent thickening and was discharged beneath the nictitating membrane as a cheesy mass, closing the eye. Infected subocular sinuses were filled with exudate. Recovering ducks suffered retarded growth and development.

Pathoanatomical signs: Hyperemia of mucous membranes of the nose and subocular sinuses; edemic lungs, with hemorrhaging in some areas; grayish yellow fibrinous deposits in air sacs, covering a significant amount of surface area; liver slightly enlarged; cardiac muscle pale. The pathoanatomical changes were less pronounced in acute cases.

The various antibiotics and chemotherapeutic preparations used for prevention and treatment did not have any effect.

Analysis of the clinical and pathoanatomical pattern as well as absence of a therapeutic effect raised the suspicion of infection caused by influenza virus.

Infected mucous membranes from the nasal passages and subocular sinuses, and brain, pulmonary, and hepatic tissue from dead and slaughtered ducklings were subjected to virological analysis. Suspensions of these organs, from which microflora were removed with penicillin and streptomycin (2,000 AU per ml), were injected into the chorioallantoic cavity of 9-10-day chick embryos. Presence of multiplying agent was determined by the hemagglutination reaction, which was performed with rooster erythrocyte suspension. The infected embryos were viewed through a candling device twice a day (morning and evening) for 72 hours.

Hemagglutinating agent 207 was isolated from organs of ducklings killed by the disease and ducklings subjected to forced slaughter. The isolated virus agglutinated rooster erythrocytes to a titer of 1:16-1:32, and in subsequent passages the titer increased to a dilution of 1:64-1:256. The virus multiplied well in the chick embryos, and it had an infectious titer (EID_{50}) of 10^{-6}.

The isolated virus was tentatively identified by the hemagglutination-inhibition test using diagnostic serums for Newcastle disease virus and Rostok and Chekhov avian influenza virus, as well as by the complement-fixation reaction using diagnostic serums for type A and A1 influenza virus and for rat viruses A/Victoria/3/75 and A/Tashkent/717/77. The latter is identical to A/Victoria/3/75 virus. The experiments established that a positive complement-fixation reaction was observed with diagnostic serum for type A and rat A/Tashkent/717/77 virus to half of the homologous titer.

We called the virus strain we isolated A/Duck/Tashkent/207/77.

The hemagglutination-inhibition test was performed with guinea pig serum in order to study the strain's sensitivity to inhibitors. It turned out to be insensitive to inhibitors.

Because type-specific serums against human, animal, and avian influenza were unavailable, the strain we isolated was identified to greater detail by the respiratory virus laboratory of the Institute of Virology imeni D. I. Ivanovskiy (with the participation of V. A. Isachenko and Ye. V. Molibog).

In cross hemagglutination-inhibition tests the isolated virus A/Duck//Tashkent/207/77 was inhibited by serums against strains with the hemagglutinin antigenic formula NZ, while in (RPNA) tests it was inhibited by serums against strains with neuraminidase antigenic formula No 1.

Our preliminary virological analyses provide the grounds for hypothesizing that enzootic influenza discovered among the ducklings was elicited by a new hybrid variant of human influenza virus with the antigenic formula NZ No 1. This strain has been recorded by the USSR Regional Influenza Center.

This hybrid is apparently the result of recombination of viruses having different biological properties in the presence of antigens NZ No 2 and NO No 1.

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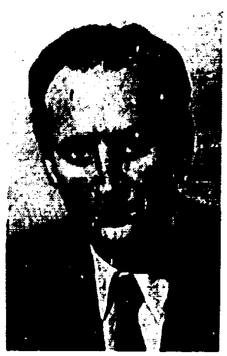
SCIENTISTS AND SCIENTIFIC ORGANIZATIONS

POGLAZOV AWARDED PRIZE BY USSR ACADEMY OF SCIENCES

Moscow VESTNIK AKADEMII NAUK SSSR in Russian No 1, 1979 p 141



[Text] The A.N. Bakh Prize for 1978 in the amount of 2000 rubles was awarded by decision of the Presidium of the USSR Academy of Sciences to Boris Federovich Poglazov, doctor of biological sciences, (Institute of Biochemistry, imeni A.N. Bakh USSR Academy of Sciences). The prize was given for his monographs "The Assembly of Biological Structures", "Mechanisms for the Assembly of Elementary Biological Structures", and "The Morphogenesis of T-Even Bacteriophages".

In the prize winning monographs B.F. Poglazov--a well known scientist who has studied problems of the molecular organization of biochemical structures--correlated results of his many years work on studying the phenomena of self assembly, on artificial reconstruction of the simplest biological structures, and on an explanation for the mechanism of genetic regulation of assembly processes. Particularly significant are his studies of bacterial morphogenesis and the mechanism of the assembly of bacterial flagella.

B. F. Poglazov was the first to succeed in dividing the corpuscles of T-even bacteriophages into their constituent components and to study their composition and physicochemical properties. He was also the first to artificially reconstruct the individual elements of bacteriophages. Electron microscopy studies using the methods of optical diffraction and circular dichroism allowed the scientist to decode the molecular organization of several structural components of bacteriophage particles and to determine the nature of structural changes occuring when host cell DNA is infected.

Studies conducted by B.F. Poglazov on protein subunit interactions and his conclusions based on these studies represent a large contribution to the theory of spontaneous self-assembly.

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SCIENTISTS AND SCIENTIFIC ORGANIZATIONS

USSR ACADEMY OF SCIENCES MAKES ORGANIZATIONAL DECISIONS

Moscow VESTNIK AKADEMII NAUK SSSR in Russian No 1, 1979 pp 142-143

[Excerpts] The decision was made to organize an Institute of Physiologically Active Substances, USSR Academy of Sciences.

The primary directions of scientific activity in the new institute are:

research, study of the structure and the relations between structure and physiological function of various synthetic and natural substances including pesticides and pheromones (which are primarily of low toxicity to warm blooded animals and man), protein-peptide substances with diverse activities including hormones and other compounds, prostaglandins, medical preparations for the treatment of infectious diseases, nervous system afflictions and other diseases, polycyclic compounds for medicine, agriculture and other fields;

development of a system for researching and estimating the effectiveness of manufactured substances with the use of biochemical and physicomathematical methods, and also the development of recommendations on practical uses of these substances in medicine, agriculture and other branches of the people's economy.

Supervision of the Institute of Physiologically Active substances is entrusted to the Section of Chemical Technology and Biological Science. The Division of Biophysics, Biochemistry, and Chemistry is charged with guidance in scientific methodology.

Doctor of chemistry I.V. Martinov has been appointed as director of the Institute of Physiologically active substances.

The Institute of Marine Biology of the Far East Scientific Center, USSR Academy of Sciences, is to organize a Far East Game Reserve at the Bay of Petr Velikiy based at the laboratory of the Marine Game Reserve Institute.

The basic directions for scientific research and activity at the game reserve are: to guarantee the preservation of restricted waters and territories, to report on sea and island biocenoses of the game reserve and their dynamics resulting from natural and anthropogenic influences, to develop scientifically based preservation and reestablishment of sea and island biocenoses of the game reserve directed towards preservation of the gene pools, to develop scientific recommendations for marine game reserve matters, to propagandize the protection of sea life through the publication of technical and popular science literature, and to develop a permanent sea nature exhibit.

The proposal of the Presidium of the far East Scientific Center, USSR Academy of Sciences, has been accepted to form a division of hydrology and hydrogeology at the Pacific Ocean Institute of Geography, FESC USSR Academy of Sciences, in the Khabarovskiy Complex Science-Research Institute, FESC USSR Academy of Sciences.

The request by doctor of biological sciences A.I. Cherepanov for release from his duties as director of the Biological Institute, SO USSR Academy of Sciences, has been satisfied.

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