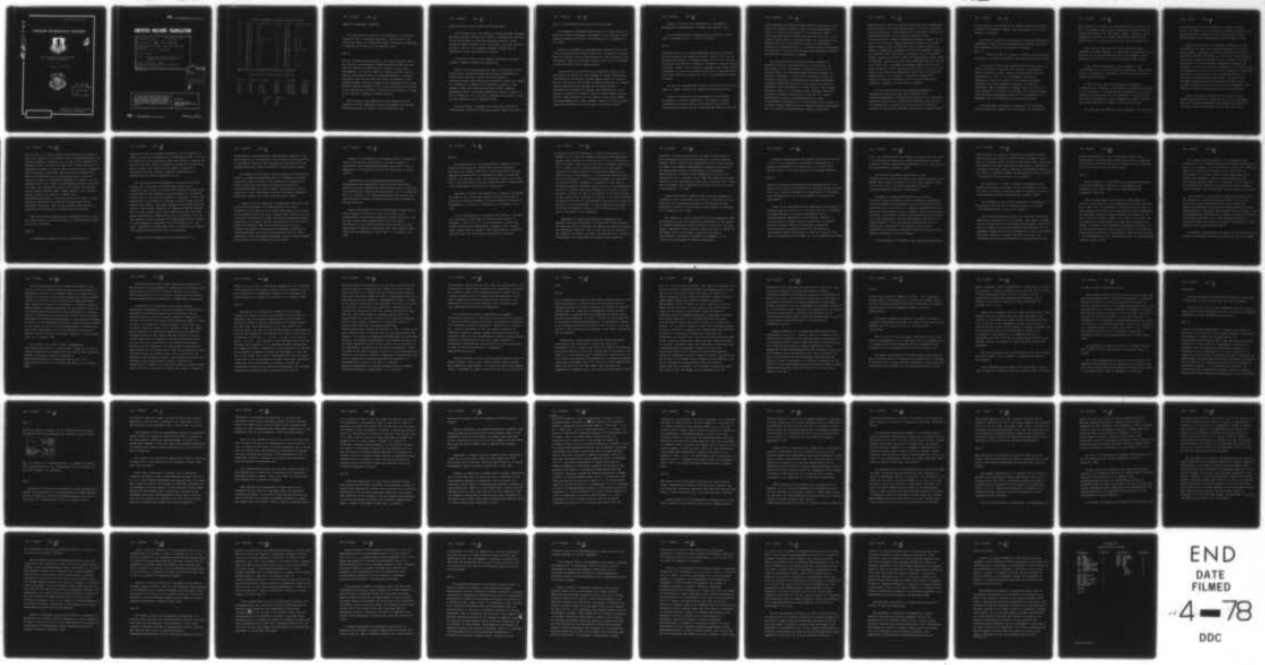
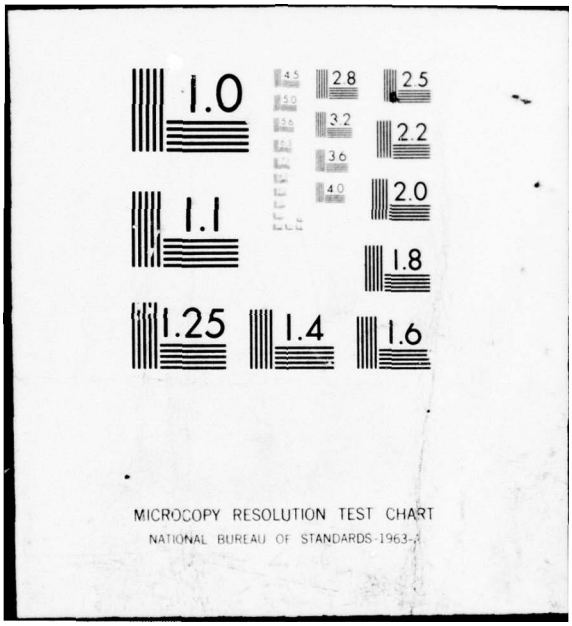
AD-A051 749 FOREIGN TECHNOLOGY DIV WRIGHT-PATTERSON AFB OHIO F/G 6/5
IMMEDIATE TYPE ALLERGIC REACTIONS (SELECTED PORTIONS),(U)
NOV 77 A D ADO, I S GUSHCHIN

UNCLASSIFIED FTD-ID(RS)T-2016-77 NL

OR
AD
A051 749



END
DATE
FILMED
4-78
DDC


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

FTD-ID(RS)T-2016-77

# FOREIGN TECHNOLOGY DIVISION


IMMEDIATE TYPE ALLERGIC REACTIONS
(SELECTED PORTIONS)

by

A. D. Ado, I. S. Gushchin

# UNEDITED MACHINE TRANSLATION

FTD-ID(RS)T-2016-77 30 November 1977

MICROFICHE NR: FTD-77-C-001493

IMMEDIATE TYPE ALLERGIC REACTIONS (SELECTED PORTIONS)

By: A. D. Ado, I. S. Gushchin

English pages: 63

Source: Modelirovaniye Zabolevaniy, Izd-vo "Meditsina", 1972, pp. 14-35.

Country of origin: USSR
This document is a machine translation.
Requester: FTD/PHE
Approved for public release; distribution unlimited.





PREPARED BY:

TRANSLATION DIVISION
FOREIGN TECHNOLOGY DIVISION
WP-AFB, OHIO.

## U. S. BOARD ON GEOGRAPHIC NAMES TRANSLITERATION SYSTEM

| Block | Italic | Transliteration | Block | Italic | Transliteration |
|---|---|---|---|---|---|
| А а | *А а* | A, a | Р р | *Р р* | R, r |
| Б б | *Б б* | B, b | С с | *С с* | S, s |
| В в | *В в* | V, v | Т т | *Т т* | T, t |
| Г г | *Г г* | G, g | У у | *У у* | U, u |
| Д д | *Д д* | D, d | Ф ф | *Ф ф* | F, f |
| Е е | *Е е* | Ye, ye; E, e* | Х х | *Х х* | Kh, kh |
| Ж ж | *Ж ж* | Zh, zh | Ц ц | *Ц ц* | Ts, ts |
| З з | *З з* | Z, z | Ч ч | *Ч ч* | Ch, ch |
| И и | *И и* | I, i | Ш ш | *Ш ш* | Sh, sh |
| Й й | *Й й* | Y, y | Щ щ | *Щ щ* | Shch, shch |
| К к | *К к* | K, k | Ъ ъ | *Ъ ъ* | " |
| Л л | *Л л* | L, l | Ы ы | *Ы ы* | Y, y |
| М м | *М м* | M, m | Ь ь | *Ь ь* | ' |
| Н н | *Н н* | N, n | Э э | *Э э* | E, e |
| О о | *О о* | O, o | Ю ю | *Ю ю* | Yu, yu |
| П п | *П п* | P, p | Я я | *Я я* | Ya, ya |

*ye initially, after vowels, and after ъ, ь; e elsewhere.
When written as ё in Russian, transliterate as yё or ё.

## RUSSIAN AND ENGLISH TRIGONOMETRIC FUNCTIONS

| Russian | English | Russian | English | Russian | English |
|---|---|---|---|---|---|
| sin | sin | sh | sinh | arc sh | $\sinh^{-1}$ |
| cos | cos | ch | cosh | arc ch | $\cosh^{-1}$ |
| tg | tan | th | tanh | arc th | $\tanh^{-1}$ |
| ctg | cot | cth | coth | arc cth | $\coth^{-1}$ |
| sec | sec | sch | sech | arc sch | $\operatorname{sech}^{-1}$ |
| cosec | csc | csch | csch | arc csch | $\operatorname{csch}^{-1}$ |

| Russian | English |
|---|---|
| rot | curl |
| lg | log |

IMMEDIATE TYPE ALLERGIC REACTIONS.

The solution to the problem of the modelling of the allergic diseases of man in experiment encounters a whole series of the difficulties which to a considerable degree determine the complexity of modelling and majority of the diseases of man.

Page 15.

Allergic illness/sickness/diseases as such are encountered only in man. The hence understandably fundamental position about the fact that the modelling of these states in animals in their entire completeness, the specific character of clinical course, with the special feature/peculiarities of organolocalization is virtually impossible. Thus, one can speak only about the modelling of the separate allergic reactions, component the pathogenetic basis of the different allergic diseases of man. In this connection very valuable is the reproduction of allergic reactions on organ/controls, tissues and the cellular structures, obtained from man.

Let us pause at the description of the classical and most frequently utilized experimental models of immediate type allergic reactions, and also on those of them, in development and

application/use of which we have its own experiment.

Active sensitization. The capacity to sensitize animal possesses any worthy antigen. However, this capacity is dissimilarly expressed for various antigens. Is high the sensitizing activity of such antigens as serous globulin of man and animals, bull serum albumin, ovalbumin, which are the classical antigens, utilized for the reproduction of the sensitization of animals.

To bacterial toxins is also specific noticeable sensitizing activity, constantly detected of guinea pigs.

Even in early investigations was shown the possibility of the sensitization of animals by defective antigens. Landsteiner and Levine (1930) sensitized guinea pigs by the isomers of para-amino-tetranil acid, bonded with proteins of horse serum. During the introduction to animals as the reacting injection of these isomers, bonded with gallinaceous/chicken globulin, appeared anaphylactic shock. To reproduce anaphylactic shock is possible by one azo compound of the guinea pigs, sensitized by azoprotein. These data were confirmed by the subsequent works.

The sensitization of animals can be reproduced also by the carbohydrates, secreted from bacteria; however, their sensitizing

activity is considerably lower than in worthy antigens.

In experimental allergology quite widely use a sensitization of animals by different allergens, for example, by the allergens of the pollen of plants and by their protein fractions (F. F. Lukmanova et al., 1967).

During the evaluation of the sensitizing capacity of one or the other antigen (allergen) should consider the form/species of animal. Thus, for instance, dogs are sensitized well by horse serum and it is considerably worse by egg protein, whereas guinea pig are sensitized to identical degree good by these antigens.

Sensitization can be reached by the different methods of the introduction of antigen into the organism of animals: subcutaneous, intracutaneous, intravenous, intraperitoneal, inhalation, enteral. Best are the first of four methods of sensitization, and for reliable enteral sensitization are required the additional methods, alleviating the absorption of antigen. Oral sensitization it was possible to reproduce in rabbits, giving tc them together with food within a month daily 30 ml of 1o/o solution of bull serum albumin (Strannegard, Yurchision, 1969). Through 2 weeks in animals, appeared reagin-like antibodies, which were being held during 4 months.

Animals, utilized for the reproduction of the classical manifestations of anaphylaxis, are guinea pigs, rabbits, dogs.

For the sensitization of guinea pig by egg protein or horse serum is sufficient $5 \cdot 10^{-8}$ - $7 \cdot 10^{-8}$ g of protein.

Page 16.

Incubation period in this case sufficiently lasting (20-25 days). With an increase in the sensitizing dose of protein to $10^{-3}$ g, the expressed sensitization is revealed already to the 7-10th day. For the reliable sensitization of rabbits is necessary a greater quantity of antigen - about 5-10 mg of protein (ovalbumin, horse serum, serous γ-globulin) on 1 kg. of the weight of body; for the sensitization of dogs - about 20-30 mg of protein on 1 kg. of the weight of the body of animal.

The state of sensitization of guinea pigs can be held of up to 2 years. In rabbits and dogs, this period is considerably shorter.

Thanks to introduction into the practice of experimental allergology of different stimulators it is possible to obtain sensitization by worthy antigen in all cases at the indicated form/species of animals. As this stimulator most widely is used the

full/total/complete Freund's adjuvant, which is the mixture of one part of the anhydrous lanolin, 2 parts of liquid petrolatum, into which it is added by 1-2 mg BTsZh on 1 ml of mixture. Before utilization the obtained adjuvant they autoclave twice with 1.5 at. during 40 minutes with an interval 24 hours. For sensitization the adjuvant mixes with the solution of antigen (in physiological solution) in relationship/ratio 1: 1, and then mixture they emulsify by agitation or with the aid of syringe before obtaining of uniform mass without the separation of phases.

The application/use of stimulators made it possible to constantly reproduce sensitization even in rats and mice. The action/effect of such stimulators of sensitization in rats as whooping vaccine and full/total/complete Freund's adjuvant, is explained, apparently, by lowering the resistance of animals to histamine. Furthermore, whooping vaccine noticeably strengthens consumption/production/generation in the rats of the cytophilic antibodies, "which sensitize fat to cell" (Keller, 1966; Binaghi, Benacerraf, 1964). L. I. Zelichenko (1969) conducted comparative research on different methods and it showed that the reliable sensitization of rats is reproduced even by the single intraperitoneal introduction 2 ml of the mixture, which consists of the equal volumes of horse serum and whooping vaccine, which contains into 1 ml $2 \cdot 10^{10}$ of microbial bodies. Reacting intravenous

administration of antigen to the 16th day causes lethal anaphylactic shock in 60o/o of the cases. Several minutes after the introduction of the challenge dose of the antigen of rat they begin to develop the restlessness/anxiety, which is gradually changed by the full/total/complete absence of reaction to painful irritants. In the first of 10-15 minutes, appears shortness of breath, is developed edema of muzzle, lugs, scrotum. After 20-30 minutes, shortness of breath is strengthened and appears the so-called abdominal syndrome: rat lie/rests, after being split on liveliness with the stretched clamps. Frequently the animal issues the characteristic squeak, which is explained, apparently by sharp spastic abdominal pains. At the end of the first hour, is possible the passage together with feces of bloody discharges. Rectal temperature falls from 38 to 34° and it is below. In the killed from anaphylactic shock rats are revealed the changes mainly in the intestine: intestine and mesentery are plethoric, noticeable hemorrhage into the wall of intestine and hemorrhagic exudation into the lumen of intestine.

For the target/purpose of the approach/approximation of experimental conditions to the natural methods of the incidence/impingement of allergens into organism, can be used other methods of sensitization, in particular inhalation. The courses of the sensitization of animals in this manner must be specially selected for the utilized allergen. The principle of method consists

of the creation of the aerosol of the aqueous solution of allergen (antigen) in the special chambers, where they place in time of the inhalation of animals.

Was recently carried out the analysis of the physicochemical and immunological properties of the anaphylactic antibodies, which were being generated during the sensitization of animals.

Page 17.

These data make it possible to evaluate, how anaphylactic a antibodies of animals correspond to the allergic antibodies of man.

Of the guinea pigs of those sensitized with worthy antigen, are formed two types of $\gamma$-antibodies with different electrophoretic mobility (Benacerraf, 1966). "Rapid" type antibodies, which have sedimentation constant 7S (7S $\gamma$-antibodies), are strictly anaphylactic, they are critical for the formation of systemic anaphylaxis and local anaphylactic reactions of guinea pigs (Benacerraf, 1966; Brocklehurst, Colquhoun, 1965) and they do not join complement in the presence of antigen. "Slow type antibodies", which also have sedimentation constant 7S (7S $\gamma_2$-antibody), join complement and do not sensitize the tissue of guinea pigs.

The anaphylactic antibodies of guinea pigs ($\gamma_1$-antibodies), which sensitize the tissues of the same animals, are thermolabile.

Their activity is retarded or is completely blocked by heating with 56°C. This property draws together anaphylactic antibodies with the allergic antibodies of man (by reagin). However, unlike the latter anaphylactic antibodies are passed through the placenta and are capable thereby to passively sensitize the tissues of fruit/fetus.

Rapid and slow type antibodies possess identical papain fragments (I and II on Porter), but they differ in properties and the structure of the heavy polypeptide chain in III fragment, which, apparently, and refers to the joining of antibody with tissues.

In mice during sensitization, also are formed $\gamma_1$- and $\gamma_2$-antibodies, and the first of them are anaphylactic. Unlike reagin of man, the anaphylactic antibodies of mice are not record/fixed by tissues during long time.

In dogs and rats, also are developed the anaphylactic antibodies, which according to electrophoretic properties are related to slow β-globulins. Of the anaphylactic antibodies of rats, much in ccmmon with reagin person. Their concentration in blood serum very low, they are thermolabile, have a sedimentation constant between 7S and 19S and sensitize the tissues of rats during long time.

All antibodies are capable of being record/fixed on tissues and

conditioning by this the sensitization of tissues of specific antigen. The capacity of the joining of anaphylactic antibodies with tissue receptors is determined that by the structural special feature/peculiarity of heavy polypeptide chain (Sorkin, 1966).

Passive sensitization. In experimental allergology is used predominantly the model of the passive sensitization of marine to guinea pig/mumps necessary to introduce 150-200 μg of pure/clean antibodies. Pure/clean antibodies, or 0.5-1 ml of the blood serum of rabbit, hyper-immunized by worthy antigen, introduce to guinea pig intravenously or intraperitoneally. Anaphylactic reaction they reproduce in 24-48 hours after the introduction of antibodies. Since the sensitization of animals in this case is realized by heterologous antibodies, it is necessary to keep in mind that they are not identical by their physicochemical nature to the anaphylactic antibodies, described above. The antibodies of rabbit, which passively sensitize guinea pig, are related to $\gamma_2$-globulins, they possess the complement-fixing property and are thermostable.

There is significant interest for the modelling of the manifestations of bronchial asthma and vivo in the intratracheal method of the passive sensitization of animals. This method was successfully used in experiments on dogs (Patterson, 1969).

The introduction of the aerosol through the intratracheal tube to a dog-recipient simply feasiblly can be repeated several times. For the passive sensitization of dogs intratracheally is used the blood serum of dog-donors whom have revealed allergic state, which arose under natural conditions (Patterson, 1969). The increased sensitivity under natural conditions meets in the high(ly)-thoroughbred (more frequent than hunting) dogs, that contact any active allergen, for example, the pollen of weed - ambrosia. In the blood serum of such animals, are contained reagin-like thermolabile antibodies. With the aid of atomizer this serum it is lead in the form of the aerosol through intratracheal tube to a dog-recipient three times in a day/every other day. Volume of the once introduced serum 3-4 ml.

For the reproduction of the reaction of bronchospasm, are used either the aqueous extract of the pollen of plant or the aqueous suspension of one-piece/entire pollen. This allergen is introduced in the form of aerosol it is intratracheal through 2 days after the last/latter procedure of passive intratracheal sensitization. Depending on the concentration of allergen and degree of the sensitization of animal, the exposure time of initial symptoms varies within the limits of several minutes after the beginning of

exhibition/exposure. Appears acute/sharp dispnoea. After stopping of the introduction of allergen, the signs of suffocation gradually disappear. They can be also easily removed by the introduction of adrenalin.

Monkeys also can be easily sensitized passive by intratracheal method (Patterson, 1969). For this sensitization is used reagin blood serum of the patients with pollinosis. The execution of the sensitization of monkeys is analogous with the intratracheal sensitization of dogs. Changes from the bronchopulmonary apparatus during the inhalation of allergen consist of increase of the frequency of respiration, decrease in the expiratory volume and the elongation of the phase of expiration. These changes begin into the first of 10 minutes after the beginning of the inhalation of allergen and gradually they disappear on the 60-120th minute after stopping of the action of allergen. The repeated inhalation of allergen either not at all causes reactions or it conditions weak reaction.

Anaphylactic shock. The variants of the clinical course of anaphylactic shock in man are different. During heavy course soon after the introduction of allergen begins sudden collapse with the loss of consciousness. Such manifestations of anaphylactic reaction as bronchospasm, skin rashes, in this case not have time to develop. In the less heavy cases the symptoms of systemic anaphylaxis appear

into the first of 10 minutes after the introduction of allergen or later. Such symptoms are a feeling of fear, restlessness/anxiety, the pulsating headache, dizziness, noise in ears, pouring perspiration. Frequently patient perceives the sharp itch of entire body with the subsequent development of nettle rash or allergic edema of Quinckes's type of different localization. The quite first manifestations of shock can be symptoms from the respiratory organs: a cough, the growing on feeling of suffocation, compression in breast, i.e., the symptoms, which are the result of bronchospasm and edema of the wall of bronchi, but sometimes also larynx. Are frequent also symptoms from the gastrointestinal tract: nausea, vomiting, the acute/sharp abdominal pains, which appear as a result of an abbreviation/contraction in the smooth muscle of intestine. Appear involuntary defecation and urination. In women appear bloody discharges from vagina. These are the symptoms, which testify to implication in the reaction of visceral smccth musculature.

Thus, the riskiest manifestations of anaphylactic shock in man are caused by drop in the blood pressure, by bronchospasm and edema of the wall of bronchi.

Page 19.

In experimental animals the leading manifestations of

anaphylactic shock are different depending on the form/species of animal, and the basic symptomatology of anaphylactic shock of man can be modeled on one form/species of animal only partially. However, the account of the most explicit pathogenetic mechanisms of anaphylactic shock in guinea pigs, rabbits and dogs makes it possible to sufficient fully model the most essential mechanisms and the manifestations of systemic anaphylaxis of man.

For the reproduction of anaphylactic shock can be used both actively and the passively sensitized guinea pigs. Lethal anaphylactic shock during the utilization of methods described above of sensitization they reproduce by the intravenous or intracardiac introduction 1 mg of protein (ovalbumin or γ-globulin). On the 1-2nd minute after the introduction of antigen in animal, appear agitated motions, wool tousles, they appear sneezing, cough, guinea pig/mumps cards by clamps muzzle; frequently are observed convulsive springs. After 2-5 more minutes animal falls sideways with tonic and clonic spasms. Inhalation is accomplished with great difficulty, with the wide opening of mouth, sometimes with interval/gaps several minutes. Is noted paralysis of sphincters, they are secreted by cal. and urine. Following this begins death. Heart is continued to be reduced still several minutes after the stop of respiration.

Antigen can be introduced also by another method: it is

intraperitoneal, intramuscularly, subcutaneously, inhalation. With such methods of the introduction of the antigen of the manifestation of anaphylactic shock, are less expressed and are noted after more lasting time interval after the contact of organism with antigen.

The symptoms of anaphylactic shock in rabbits are predominantly caused by an abbreviation/contraction in the pulmonary arterioles. Excitation, cough, the signs of the difficulty of respiration are absent. Animal falls with sharp convulsive motions and the liberation/excretion of feces and urine. Through several minutes begin death. Unlike guinea pigs, irregular respiratory movements in rabbits are retained for a while after the stop of heart.

In dogs the driving mechanisms of anaphylactic shock are a drop in the arterial pressure, the redistribution of the blood, implication in the reaction of the smooth musculature of the vessels of the abdominal cavity and liver. Through several minutes after the intravenous introduction of antigen dog reveal restlessness/anxiety, excitation, in it appears vomiting, are secreted by cal. and urine, painted by the blood. Then sets in comatose state, dog falls sideways, the muscles of extremities are weakened, respiration is attenuate/weakened, from mouth is secreted the foam. With lethal anaphylactic shock the weakness progresses, again can appear vomiting and diarrhea and with convulsions it begins death.

Whatever the differences in the manifestations of anaphylactic shock of the different form/species of animals, important in pathogenetic sensa disturbance/breakdowns caused first of all by implication in the reaction of one or the other groups of smooth muscles.

Anaphylaxis of the isolated/insulated smooth-muscle organ/controls most successfully models the basic pathogenetic mechanisms of such widespread and risky manifestations of allergy in man as anaphylactic shock and bronchial asthma, and it is convenient model for research on the ccmmon/general/total laws governing the sensitization of different tissues and pathochemical mechanisms of allergic reactions.

Passive sensitization of the isolated/insulated smooth-muscle organ/controls. The majority of facts was obtained on the organ/controls of guinea pigs (intestine, uterus, light/lung), and are only unitary report/communications about the passive sensitization of the isolated/insulated organ/ccntrols of other form/species of animals, in particular rats, mice (Saikawa, 1965), monkeys and man (Tollockson, Frick, 1966; I. S. Gushchin et al., 1968).

Page 20.

The sensitization of the organ/controls of guinea pig can be reached by the antibodies, obtained only from the determined form/species of animals. The greatest sensitizing activity possess homologous antibodies and the antibodies of rabbits, considerably smaller - the antibody of man. The antibodies of horse, bull, sheep, birds in any concentrations are not caused the passive sensitization of the smooth-muscle organ/controls of guinea pig.

Dependence of passive sensitization on a quantity of antibodies. The degree of the passive sensitization of the organ/controls of guinea pig by rabbit antibodies with time constant of incubation is proportional to a quantity of antibodies (I. S. Gushchin et al., 1968).

For the reproduction of the passive sensitization of intestine and light/lungs, is sufficient an extremely small quantity of antibodies - about 0.1 μg on 1 ml of liquid/fluid in which they incubate organ/control (Brocklehurst, Colquhoun, 1965). Maximum effect appears with an increase of the concentration of antibodies 100-1000 times. With the greater concentration of utilized

antibodies, begins the inhibition of passive sensitization (I. S. Gushchin et al., 1968). The anaphylactic reaction of organ/control appears during fixation in it less than 0.1-0.3 μg of antibodies on 100 mg of tissue. With the aid of the rabbit antibodies, mark/tagged $I^{131}$, is shown, that for the reproduction of the anaphylactic reaction of the small intestine of guinea pig is sufficient the fixation 0.6 μg of antibodies on 1 g of damp/crude tissue and even 0.02 μg of antibodies on 1 g of damp/crude tissue. The difference in the obtained results, apparently, is explained by the degree of purification/cleaning antibodies. When the force of the anaphylactic reaction of pulmonary tissue was considered in a quantity of freed histamine, it turned out that to one molecule of the fixed/recorded antibody comes several thousands of molecules of the freed histamine. In actuality this numeral is considerably more, since by no means all molecules of the fixed/recorded in tissue antibodies participate in the release of histamine, since not all of them are located near cells - the sources of this substance.

Dependence of passive sensitization on temperature. The antibodies of those form/species of animals which do not sensitize the organ/controls of guinea pig, are adsorbed on them both good and the sensitizing antibodies. That the fact that for the creation of sensitization is required the special form/species of the "fixation" of antibodies, show experiments on a change in the temperature

conditions of passive sensitization. the speed cf the joining of rabbit antibodies by the ilium of guinea pig it virtually remains constant during a change in the temperature from 20 to 37°C, whereas the degree of the passive sensitization, evaluated in the value of the anaphylactic reaction of organ/control, is strengthened proportional to an increase in the temperature in the period of sensitization (I. S. Gushchin et al., 1968). The adsorption of rabbit antibodies by the light/lungs of guinea pig is increased only 2 times with an increase in the temperature from 0 that 37°C, but the speed of sensitization - 15 times.

Maximum sensitization reaches at 40°C (with time constant of incubation), but at temperature above 40°C it is decreased, which is bonded with the disturbance/breakdown of the mechanism of quite anaphylactic reaction or with decrease of the fixation of antibodies (I. S. Gushchin et al., 1968).

The dependence of passive sensitization on the temperature makes it possible to assume the possibility of implication in this process of different fermentation systems. However, it turned out that many agents, suppressing cellular enzymatic reactions, such as cyanide (10 millimole), azide (50 millimole), fluoride (5 millimole), dinitrophenol (2 millimole) and phenol (10 millimole), do not influence the develcpment of passive sensitization.

Duration of sensitization. With optimal temperature conditions (37-38° C) for achievement of the maximum of sensitization is required the determined time interval from 1 to 3 hours whose duration depends on the concentration of the utilized antibodies.

Page 21.

Duration of incubation for developing the sensitization has even greater value, than the concentration of antibodies (Feigen, Nielsen, 1966). So, a 1000-fold decrease in the concentration of antibodies can be compensated for by a 45-fold increase in the duration of sensitization.

Effect of ions and $pH$ on passive sensitization. The presence of inorganic ions (Na, Mg, CA, Cl, K, $SO_4$, $HPO_4$, Br) not is compulsory for accomplishment of the passive sensitization of the isolated/insulated smooth-muscle organ/controls. Moreover, in ion-free medium in isotonic solution (solutions of saccharose, dextrose, fructose, lactose and arabinose) sensitization occur/flow/lasts considerably faster, but the addition of electrolytes retards sensitization. In this case, does not have a value the form/species of the added ion. On the light/lungs of guinea

pig, it was reveal/detected that during a decrease in the ionic force of the sensitizing solution was increased the adsorption of immune γ-globulin; however, strengthening sensitization in this case did not begin (Brocklehurst, Colquhoun, 1965).

The process of the passive sensitization of the isolated/insulated bowels of guinea pig with rabbit antibodies is not changed during change of $pH$ from 8.2 to 5.6. But if $pH$ they change with the aid of $CO_2$, then begins inhibition or even the full/total/complete blockade of sensitization.

The degree of passive sensitization can be raised by introduction into the medium in which is sensitized organ/control, ureas. The amplifying action/effect of urea is developed with the concentration of 0.16 moles, and it reaches the maximum with of 0.8 moles. The action/effect of urea is effective only in the presence of antibodies. The treatment of isolated/insulated intestine with urea to or after conducting of passive sensitization is not led to the described effect. At the same time the thorough washing of the organ/control, sensitized in the presence of urea, is not remove/taken the intensive anaphylactic reaction. The nature of the described phenomenon thus far is unclear.

On the properties of antibodies, which sensitize smooth-muscle

organ/controls. The capacity to sensitize the isolated/insulated organ/controls possess not all types of known antibodies. Works of Ovary and coworkers (1963) and White and coworkers (1963) showed that the antibodies of guinea pigs with the coefficient of sedimentation 7S belong to two types: some of them with electrophoretic mobility in the region of $\gamma_1$-globulin possess the sensitizing activity, of others - with mobility in region there is no $\gamma_2$-globulin.

For guinea pig $\gamma_1$- globulin possesses incomparably greater sensitizing activity with respect to the organ/controls of guinea pig, than $\gamma_2$- globulin, whereas the degree of adsorption of each in tissue virtually identical (Brocklehurst, Colquhoun, 1965).

The antibodies of man with the coefficient of sedimentation 7S are capable of sensitizing the tissues of guinea pig, but for $\gamma_1$-macroglobulins does not have this capacity.

The isolated/insulated cuttings off of the small intestine of monkey can be sensitized by the antibodies, which relate to classes IgA and IgG blood serum of the patients with pollinosis, whereas the bowels of guinea pig can be sensitized only by antibodies IgG. The antibodies, bonded with IgA, are thermolabile, and their sensitizing activity is decreased by heating with 56°C for 4 hours. IgA sound/healthy people's serum suppresses the sensitization, caused

cnly by IgA-antibodies, and IgG sound/healthy people's serum remove/takes the sensitization, caused by both types of antibodies. In human serum are present at least two fractions of the antibodies one of which (IgA) can be referred to reagin.

Page 22.

Further research on the mechanism of the passive sensitization of the small intestine of monkeys by the antibodies of man (Kobayashi, et Al, 1967; Wicher et Al, 1968) confirmed and supplemented these data.

After the publication of the materials, which testify to an existence of the new type of the immunoglobulin of class E (Ishizaka et Al, 1966, 1967), not detached it is earlier from other types of immunoglobulins, was studied the interrelation of this immunoglobulin and sensitizing capacity of human antibodies with respect to the small intestine of monkeys (Wicher et Al, 1968). It was shown, that the exhaustion of immunoglobulin E in human sera suppresses their capacity to sensitize the smooth-muscle preparation of monkeys, and thereby was experimentally substantiated the bond of the sensitizing antibodies with this type of immunoglobulin. These antibodies proved to be high-sensitivity to the processing/treatment by mercaptoethanol (Buckley, Metzgar, 1966).

As a result of detailed research on the mechanism of the sensitization of the isolated/insulated smooth-muscle organ/controls of man (I. S. Gushchin et al. 1968, 1970; A. D. Ado et al., 1969) was shown, that the allergic antibodies of man, critical for the sensitization of the smooth muscles of man (small intestine and bronchi) were analogous to reagin. These antibodies are also thermolabile, arrange/located in the same zone of the fractions of the serum proteins, obtained by gel filtration through Sephadex I-200; are bonded with the same types of immunoglobulins, as reagin.

Characteristic of an anaphylactic abbreviation/contraction in the smooth-muscle organ/controls and the effect on it of some factors. The special feature/peculiarity of an anaphylactic abbreviation/contraction in the various organ/controls is the time characteristics of shortening answer/response under the effect on the isolated/insulated organ/controls of chemical stimulators (histamine, acetylcholine, serotonin, etc.). This peculiarity consists in relatively lasting latent period, the period of growth/build-up and decrease in the abbreviation/contraction.

Of different organ/controls the duration of the latent period of anaphylactic abbreviation/contraction varies from 10 to 60 seconds.

The temporary and quantitative characteristic of anaphylactic abbreviation/contraction most completely studied in the isolated/insulated small intestine of guinea pig. For the extent/elongation of small intestine, the sensitivity of muscle to histamine, acetylcholine, serotonin, ions K and to specific antigen is dissimilar; are most sensitive to these substances the extremital sections of the ilium.

During the study of the anaphylactic reaction of smooth-muscle organ/controls, one should bear in mind that after the addition of antigen is developed specific desensitization of organ/control for antigen. Therefore for research on the effect of different factors on anaphylactic abbreviation/contraction, usually are used the adjacent cuttings off of small intestine of one and the same guinea pig. Some researchers dispute the value of similar experiments, since, according to their data, the separate cuttings off of small intestine of the one and the same sensitized guinea pig answered by dissimilar by value reaction to the defined doses of both antigen and the histamine, and acetylcholine. However, in this work, and in the majority of the investigations, dedicated to research on anaphylaxis of smooth-muscle organ/controls, is used the isotonic method of the record of abbreviation/contraction. As showed special investigations, this method really is not absolutely accurate for the quantitative evaluation of the shortening answer/response of smooth muscle, since

the isotonic shortening of muscle can be maximum under the submaximum force of stimulation. From similar deficiencies is free the method of isometric record, which makes it possible to obtain a strict quantitative characteristic of stress level, developed with muscle in response to the action/effect of the various stimulating agents.

Page 23.

Therefore introduction into the experimental practice of the study of the anaphylactic reaction of the smooth-muscle organ/controls of the isometric method of record makes with especially valuable the obtained in this case results (I. S. Gushchin, 1966-1969).

The amplitude of anaphylactic abbreviation/contraction, measured under conditions of isometric or isotonic record, is increased proportional to an increase in the dose of antigen, thus far is not reached the maximum of abbreviation/contraction. The maximum force of an anaphylactic abbreviation/contraction in the cut (1.5-2 cm) of small intestine of guinea pig under conditions of isometric record is approximately 6 g. The quantity of antigen, necessary for achievement of maximum abbreviation/contraction, in different cases is different. This depends both on the form/species of the utilized antigen and on the degree of sensitization.

Is establish/installed the bond between the mechanical and electric activity of smooth-muscle cells during the development of anaphylactic reaction (I. S. Gushchin, 1966). It is shown, that an increase in the muscle tension during anaphylactic reaction precede changes in the electric activity of cellular diaphragm/membrane. These changes are expressed in a decrease in the membrane potential (depolarization of diaphragm/membrane) and the frequency increase of the spontaneous action potentials, which characterize the rhythmic activity of smooth-muscle cells and which activate their shortening mechanism. The quantitative manifestation of such changes corresponds to the force of the shortening reaction of muscle. Discovered changes in the electric activity of the diaphragm/membrane of smooth-muscle cells during anaphylactic reaction are analogous to the changes, observed under the different stimulating influences on smooth-muscle cells (I. S. Gushchin, 1969).

The duration of the latent period of anaphylactic abbreviation/contraction to a certain extent depends on the dose of utilized antigen and is decreased with its increase. The significant duration of the latent period of anaphylactic abbreviation/contraction is determined that by time of the penetration of antigen to the reacting structures (I. S. Gushchin, 1969).

Abbreviation/contraction strength during the utilization of a maximum dose of antigen somewhat is increased with an increase in the temperature from 25 to 40°C and with an increase in the initial muscle tension from 0 to 2.5 g, which is bonded with an increase in the common/general/total excitability of smooth-muscle preparation.

An increase in the temperature in the which surrounds organ/control liquid/fluid to 45°C makes possible the reproduction of anaphylactic abbreviation/contraction. the sensitized uterus, processed during 5 minutes in Ringer's solution at temperature of 45°C and then placed into solution with temperature of 37°C, it does not react to the addition of specific antigen. In this case, the shortening capacity of muscle is not disturbed. Normal light/lungs and the bowels of the guinea pig, sensitized with the antibodies, thoroughly heated at 45°C, reacted with the addition of specific antigen. When first were heated the indicated organ/controls with 45°C, and then they treated them by the sensitizing antibodies, reactions were not discovered. The inactivation of anaphylaxis of smooth muscles can be obtained and in vivo. The organ/controls of the sensitized guinea pig, heated to 43°C, are not reduced with the addition to them of specific antigen. This process of inactivation is reversible, and the anaphylactic reaction of smcoth-muscle organ/controls is reduced in 24 hours after heating of animal. During heating occurs the inactivation of scme factor, which is located in

tissue. It is not clear, is this factor necessary for the containment cf the molecules of antibodies in cells or for the realization of the very mechanism of the anaphylactic reaction, which associates the addition reaction of antigen with antibcdy (I. S. Gushchin, 1968).

Page 24.

Reproduction of an anaphylactic contraction in the small intestine and uterus of guinea pigs (reaction of Schulz-Daily). In experiment they use guinea pigs approximately 250 g in weight. Animals are killed by means of bloodletting from carotid arteries. The cuts of the ilium approximately 2 cm in long secrete near ileocecal angle. Then each cut are cautious washes in liquid/fluid of Krebs of room temperature. The composition cf Krebs' liquid/fluid (in millimcles): Na are 137.4, K - 5.9, Mg - 1.2, Ca - 2.5 Cl are 134, $H_2PO_4$ - 1.2, $HCO_3$ - 15.5, glucose is 11.5. From both ends of the cut, reinforce silk ligatures. All the manipulations with organ/control are conducted in Krebs' liquid/fluid at room temperature. In the case of the setting of reaction on uterus into experiment, is taken one horn of the uterus. Dale (1913) in experiments were used the virgin guinea pigs of the outside period of estrus. Preliminary treatment of guinea pigs with estrogen makes it possible to increase the sensitivity of uterus in the reaction of Schulze-Daily. The prepared smooth-muscle preparation then is placed into tray for the

isolated/insulated organ/controls. Tray is glass cylinder with two offtakes: below and in upper section. Volume of the tray between these offtakes 5-10 ml. Through the lower offtake feeding solution enters tray, and while through the upper it escape/ensues from it. To preferably use constant perfusion (approximately 2 ml/min in speed). By one ligature organ/control they reinforce at the bent in the form of a hook end of the hollow glass small tube, immersed/inserted together with organ/control into tray. Through this same the small tube into tray, enters oxygen or carbogen. Another end of the organ/control they reinforce on lever for the record of shortening activity under isotonic conditions or on sensor for record in iscmetric mode/conditions. Last/latter method more adequate/approaching. Feeding liquid/fluid (to 37°C) is heated in the water bath into which is submerged the tray with organ/control. The methods of the recording of abbreviation/contractions can be the most different: on kymograph, photoelectric, from oscilloscope face, ink. The tested substances, added to organ/control, must be dissolved on the same feeding liquid/fluid and are heated preliminarily to 37°C. The volume of the added solutions is about 0.2 ml. Are described the methods of the automatic addition of substances to organ/control. The value of anaphylactic abbreviation/contraction expresses or in percentages with respect to the value of a maximum abbreviation/contraction in the same preparation to any standard stimulator (histamine, acetylcholine), or in histaminic

(acetylcholine) equivalents. For this, after the establishment of the excitability of smooth-muscle preparation at fixed level, they build the calibration curve of the response reactions of muscle for the increasing concentrations of histamine (or acetylcholine), and the value of anaphylactic abbreviation/contraction expresses in the concentration of the utilized substance, which gives the same of value abbreviation/contraction as anaphylactic.

For conducting the passive sensitization the prepared smooth-muscle preparation is placed into test tube with the solution of rabbit antibodies against any antigen. Antibodies are dissolved in 10o/o (isotonic) solution of saccharose. Sensitization is conducted for 2 hours at temperature of 37°C and constant saturation of solution by oxygen or carbogen. Maximum sensitization under these conditions begins with the concentration of rabbit antibodies against ovalbumin of approximately 50-100 μg/ml. After conducting of sensitization organ/controls they wash clean in Krebs' liquid/fluid and they use for the reproduction of anaphylactic abbreviation/contraction.

Passive anaphylaxis of the isolated/insulated small intestine of monkey (Avdeyeva, 1969). Of the ilium of monkeys (Macacus rhesus) they prepare cuts approximately 2 cm in long and after their washing retain to experiment in Krebs' liquid/fluid with 4°C not more than 48

hours.

Page 25.

Passive sensitization realizes by the methcd, described above. As the sensitizing material is used the serum cf the patients with the increased sensitivity to pollen allergens (to allergens of ambrosia, timothy grass, the hedgehcgs of polythalamcus, fescue). Maximum sensitization is achieved during the utilizatien of reagin sera in breeding/culture/dilution of 1:1000 - 1:10 000. for the reproduction cf anaphylactic abbreviation/contraction, is used specific allergen in the breeding/culture/dilution which is not less than 10 times the more than toxic concentratien, determined on the control unsensitized cuts of intestine.

Reproduction of bronchospasm during the isclated/insulated preparation of the light/lungs of guinea pig. Guinea pig approximately 300 g in weight they drive in by means of bloodletting from carotid arteries. They reveal the neck part of the trachea, they insert in it the direct/straight tracheotomy tube approximately 2.5 cm in length. For obtaining the preparation of the isolated/insulated light/lungs of fin/edge, they cut on the line of their compound with breast bone and the latter they remove. Then they sever the esophagus, the downcoming part of the aorta and spinal column at the

level IX - XII of thoracic vertebrae. After opening pericardium, they uncover the point of emergence of aorta and pulmonary artery. Aorta they tie up, and pulmonary artery they reveal and insert in it cannula (perfusion) in the direction from heart. Pulmonary vascular system washes through this cannula in feeding liquid/fluid approximately 5 ml/min in speed. The head of heart is cut off and remove the part of the ear of left auricle, which facilitates the passage of the perfusing liquid/fluid through the tissue of light/lung. Then the prepared preparation of light/lungs is placed into special apparatus. It is the pressurized/sealed occluded cylinder from the organic glass with a height of 25 cm and with a diameter of 9 cm. Within cylinder is placed the infundibulum bellying of which is directed to cylinder cover. Infundibulum facilitates the collection of the perfusion liquid/fluid, which ebbs from light/lungs. In cylinder cover, there are two conclusion/derivations, on one of which by transitional rubber tube they reinforce trachea, and on other - pulmonary artery. The preparation of light/lungs thus is suspended above infundibulum. Through the trachea into light/lungs, enters the air during the "respiratory/breathing" motions of light/lungs, which are created by rhythmic changes of the air pressure in the chamber with a frequency of approximately 60 per minute. Through the pulmonary artery is fed the perfusion liquid/fluid with a velocity of of 1-2 ml/min, saturated by oxygen and heated to 37°C. The chamber with the preparation of the

isolated/insulated light/lungs is placed into the water bath, which provides the constancy of temperature conditions. Feeding liquid/fluid consists of two parts by volume of the liquid/fluid of Krebs and one part of the solution of polyglucine. In the bottom of the chamber, is located the out-gate through which escape/ensues perfusion liquid/fluid at the torque/moment of a pressure increase of air in the chamber. Tested substances are introduced into the current of perfusing liquid/fluid, which enters the pulmonary artery. "respiratory/breathing" motions record with kymograph with the aid of a Marey capsule, connected through the T-connection with intratracheal cannula.

Passive anaphylaxis of the isolated/insulated smooth-muscle organ/controls of man (I. S. Gushchin, 1969; G. V. Poryadin, 1969). Experiments are conducted on the isolated/insulated preparations of the ilium and bronchi, obtained within the limits macroscopically of the not changed tissues during different operations. For the preparation of smooth-muscle preparation from fractional bronchi, is used the method of the preparation of the "spire" of bronchi. For this, in the purified from the surrounding tissue bronchus they insert the glass rod according to the diameter of bronchus and they conduct by razor spiral-shaped cut/section along the length bronchus, secreting the fine/thin strip with a width of 3-4 mm and with a length of 30-40 mm.

Page 26.

In this case, succeeds in making, as a rule, 1 1/2-2 revolutions around bronchus. The secreted spire they bind into annulus, and on the side, contradictory/opposite bonded to ligatures, they reinforce another ligature for the subsequent fixation in tray for organ/controls.

From smaller (segmental) bronchi it is possible to prepare a preparation-chain/network, for which the removed bronchi are cut into separate annuli and they bind them (on 4-5 annuli) into chain/network.

From the ilium of man, they prepare longitudinal strips whose length is approximately 1.5-2 cm and whose width is approximately 0.5 cm. Prepared smooth-muscle preparations can be retained in Krebs' liquid/fluid to the experimental setup for 24-72 hours with 4°C.

The passive sensitization of the smooth-muscle preparations of man is conducted just as the smooth-muscle organ/controls of monkeys. As the serum, which contains allergic antibodies, is most better used blood serum of the patients with the increased sensitivity to the

pollen of ambrosia. Maximum sensitizaticn at temperature of 37°C and the duration of 2 hours is achieved in this case with use of a serum in breeding/culture/dilution 1;10 and it is below. For the reproduction of anaphylactic abbreviation/contraction, use an allergen of the pollen of ambrosia in final concentration (on nitrogen) 30 μg in 1 ml.

Passive skin anaphylaxis (PKA) of guinea pig successfully models immediate type skin allergic reactions, which take place, with increase in the permeability of skin vessels, edema and hyperemia of skin. An increase in the permeability of skin of guinea pigs is difficult to note without the utilizaticn of staining substances. The first successful attempt at the intravenous introduction of color/paint for demonstration of the immediate type of the anaphylactic reaction of the skin of guinea pigs was carried out by Ramsdale. However, only later many years this mcdel received wide acceptance.

For the reproduction of PKA, most frequently they use the guinea pigs with a weight of 250 g. Results tc conveniently estimate on white animals.

During manipulation with animals, one should observe special care in order not to cause in them stress reaction, since the latter

can make impossible reproduction of PKA.

Several hours before the setting of reaction pre-cut wool they shave off by electric razor. During the utilization of a depilatory skin must be treated by it for 24 hours before the setting of reaction. The skin of back is most adequate/approaching, since on it are obtained the more distinct reactions. Intracutaneous injections make from both sides at a distance 1.5 cm from center line and at a distance 1.5-2 cm from each other, so that from each side it is accomodated on 3-4 sample/tests. Intracutaneous sample/tests fulfill with the aid of the combined (tuberculin) syringe by the capacity of 1 ml. Fine/thin needle is introduced into skin on 1-2 mm. Volume of the introduced material 0.1 ml. Intravenous introduction to conveniently conduct into the subcutaneous vein of front/leading clamps.

In the majority of cases the reaction is considered through 30-45 minutes after the introduction of the resolving pose of antigen.

As a rule, maximum reaction reaches on the 10th minute. For alleviating the evaluation of the weak reactions of animals, they clog, skin is remove/taken and is measured the diameter of reaction on the reverse side of skin. If necessary can be used the

illumination.

Since the reactivity of guinea pigs is dissimilar, in each set of experiments, one should include not less than 4-6 animals.

Sensitization of skin by rabbit antibodies. The blood serum of rabbit, which contains antibodies, is used in the dilute or undiluted form/species and they introduce intracutaneously.

Page 27.

Antigen is introduced intravenously in volume 0.5 ml. Antigen is dissolved in 1o/o solution of Evans' bluing, prepared in physiologic salt solution. If is used the undiluted serum or its breeding/culture/dilutions less than 1:100, then antigen one should introduce in 5-7 hours after the intracutaneous introduction of antibodies. The low breeding/culture/dilutions of serum can by themselves cause the stimulation of skin and an increase in the permeability. In these cases color/paint one should introduce for 30 minutes before the intravenous introduction of antigen. If during the first 30 minutes of no reaction on the spot of the introduction of antibodies it appears, i. e., confidence in the fact that the reaction after the introduction of antigen is specific. During the utilization of higher breeding/culture/dilutions of serum color/paint

and antigen can be introduced simultaneously without fears. Is absolutely necessary the determined latent pericd between the introduction of antibodies and antigen during which to occur the fixation of antibodies in skin. In the first of 25-30 minutes after the introduction of antibodies reaction it is nct possible to reproduce generally. Most justified is time interval between the introduction of antibodies and antigen of approximately 3-6 hours.

An increase in the permeability of the vessels with PKA is developed very rapidly and is not retained for a long time. Thus, the color/paint, which emerges from vessels during an increase in their permeability, makes attained the evaluation of reaction. From the aforesaid it is understandable that the color/paint must be introduced either before the injection of antigen or it is simultaneous with it, but on no account after injection, since an increase in the permeability of vessels during this reaction passing.

If model of PKA is used for the detection of antibodies, then it is necessary to use antigen in significant excess. It was shown, that the quantity of antigen, necessary for the reprcduction of the maximum PKA, is inversely proportional to a quantity of antibodies. The necessary quantity of antigen for the reproduction of the maximum PKA is directly proportional to the value of the molecule of antigen (Table 1).

Since the significant excess of antigen does not inhibit the reaction of the determination of antibodies into PKA, it is possible to use an antigen in the quantity, which exceeds 10-100 times that necessary for maximum reaction.

Data Table 1 can be disseminated to other antigens.

PKA can be reproduced by worthy antigens or polyvalent haptene/haptens. Monovalent haptene/haptens do not reproduce PKA, but they can inhibit reaction to reacting administration of full/total/complete antigen, if the latter is introduced soon after the intravenous injection of monovalent haptene/hapten. These information were obtained with antibodies against lactoside, D-phenyl-( para-azobenzoylacetate), dinitrophenyl and trinitrophenyl. In the reaction of the inhibition when are used low-molecular haptene/haptens, PKA one should estimate not later than 15 minutes after the introduction of full/total/complete antigen. For the reproduction of threshold reaction, it is sufficient about 0.003 μg of antibodies (on nitrogen) in such a case, when is used hyperimmune serum. Somewhat greater quantity of antibodies is required in the case of applying the weaker sera, since normal γ-globulin, competing with the molecules of γ-globulin of antibodies for tissue receptors, slows down PKA.

Table 1.

Quantity of antigen, necessary for the reproduction of PKA during utilization 0.025 μg of antibodies (on nitrogen) (on Ovary, 1964).

| (1) Антиген | (2) Молекулярный вес | (3) Количество антигена, воспроизводящее ПКА, мгк |
|---|---|---|
| (4) Рибонуклеаза | 13 683 | 0,63 |
| (5) Яичный альбумин | 40 000 | 10,00 |
| (6) Бычий сывороточный альбумин | 70 000 | 31,00 |
| (7) Гемоцианин | 6 500 000 | 100,00 |

Key: (1). Antigen. (2). Molecular weight. (3). Quantity of antigen, which reproduces PKA, μg. (4). Ribonuclease. (5). Egg albumin. (6). Bull serum albumin. (7). Hemocyanin.

Page 28.

Sensitization of skin by the antibodies of other form/species of animals. The antibodies not of all form/species of animals sensitize the skin of guinea pig. While for the sensitization of the skin of guinea pigs it is required about 0.003 μg on nitrogen of the

antibodies of rabbit and guinea pig, then for sensitization by the antibodies of mice them it is necessary 10-25 times more, but for sensitization by the antibodies of rats - 50-100 times it is more.

The antibodies of man with sedimentation constant 7S (molecular weight 160 000) are capable of sensitizing the skin of guinea pig, but high-molecular antibodies (molecular weight 1 000 000) with sedimentation constant 19S and more rapid electrophoretic mobility do not possess this capacity. Reagin, apparently, do not sensitize the skin of guinea pig.

The absence of the sensitizing capacity with respect to the skin of guinea pig is reveal/detected of the antibodies of horse, cows, goat, sheep and hens.

PKA of other form/species of animals. PKA is sufficiently constantly reproduced on mice by the antibodies of rabbit and mice. It is it is most adequate/approaching for these purposes of the mouse with a weight of 20-25 g. The quantity of antibodies, sufficient for the reproduction of weak reaction, in this case must be 25-50 times greater than for obtaining PKA of guinea pig; the volume of the antibodies, introduced intracutaneously to mice, must be not more than 0.05 ml. Antibodies are retained in the skin of mice shorter time than in guinea pigs; optimal time interval between the

introduction of antibodies and antigen is 1-1 1/2 hours. The intravenous introduction of antigen in volume 0.25 ml is realized either into caudal vein or intc the dorsal vein of floor/sex term. Reaction in mice is developed more slowly than in guinea pigs. In the mechanism of PKA of mice, apparently, takes part anaphylotoxin.

PKA can be also obtained in rats. It is better to use male rats with a weight of 150-200 g. For reproduction of weak reaction in rats, is required 50-100 times of the more antibodies, than in guinea pigs. An interval among the introduction of antibodies and antigen also must be smaller than the case of the reproduction of PKA of guinea pigs. In rats in contrast to the guinea pigs antibodies of horse are capable of sensitizing skin.

The investigations, carried out on rabbits, showed that and of this form/species of animals can be reproduced PKA by homologous antibodies (Zvaifler, Bekker, 1966). Ovary (1958) and Layton (1961) were obtained PKA in hamsters and monkeys.

Other methods of the reproduction of PKA. Antigen can be introduced into skin into the same place, where were previously introduced antibodies. In this case is used the same principle, as upon the setting of the reaction of Prausnits-Kyustner on people. This method gives good results. However, during its application/use

it is necessary to keep in mind some facts. Is expedient the place of the introduction of antibodies to mark, so that then it would be possible to introduce the antigen into the same place and as control into adjacent region. Color/paint is injected intravenously directly before the intracutaneous introduction of antigen. This method proves to be useful when are tested very small quantities of antigen (for example, 0.005 μg of egg albumin). However, if a quantity of antibodies, introduced intracutaneously, 50 times and more exceeds the quantity of antibodies, necessary for the reproduction of the weak PKA, then for obtaining positive reaction it is necessary to increase the dose of antigen. Certain quantity of antibodies remains uncombined with the tissues of guinea pig, and free antibodies, reacting with the introduced antigen, they eliminate it from the subsequent anaphylactic reaction.

Page 29.

Active skin anaphylaxis. In the actively sensitized animals small quantities of antigen, introduced intracutaneously, causes the distinct skin anaphylactic reaction which is similar to the reaction, reproducible on the passively sensitized guinea pigs. As controls when conducting of this reaction to the same animal intracutaneously is inserted physiological solution, and to another (unsensitized) animal - antigen. Color/paint in these cases is injected

intravenously directly before the introduction into the skin of antigen.

Cellular anaphylaxis. The cellular mechanisms of immediate type allergic reactions are investigated on the classical models of the anaphylactic reaction of the cells: the adipose cells connective tissue, leukocytes and first of all of the basophils of blood. These cells play the role of target cells in immediate type allergic reactions.

Anaphylaxis of adipose cells. The adipose cells of connective tissue are one of the basic sources of the histamine and other biologically active materials, which are free/released in the second, pathochemical stage of allergic reactions (A. D. Ado, 1959).

Research on anaphylaxis of adipose cells is usually conducted on cells of the light/lungs of guinea pig, rich in histamine and the slow-operating substance of anaphylaxis, skin of rats and mice, containing a large quantity of histamine and serotonin, mesentery of guinea pigs, rats, hamsters, on the adipose cells, secreted from the abdominal cavity of rats. The latter are the most convenient object for research on anaphylaxis of adipose cells, since experiments in the washed clean adipose cells reduce to minimum the effect of one or the other humoral factors (Zelichenko, 1969).

9) Obtaining peritoneal suspension. Under deep ether/ester narcosis to rat are intraperitoneally introduced 6-8 ml that heated to 37°C solution of hemocele. Composition of the solution: gelatin - 8.5 g, NaCl - 8.5 g, KCl - 0.38 g, $CaCl_2$ - 0.7 g, the distilled water to 1 l. During 1-2 minutes is conducted the light/lung massage of the front/leading wall of stomach. Then on the center line of stomach is made the cut/section with a length of 1.5-2 cm through all layers. Rat carefully is turned over by the abdomen downward so, in order to from the obtained opening/aperture hung the loop of intestine. Peritoneal liquid/fluid is collected drop by drop, that flow from intestine, into the test tube, moistened by heparin. Suspension carefully is mixed with anticoagulant and during experiment retain in thermostat with 37°C. The liberation/excretion of adipose cells from the remaining components of peritoneal suspension is conducted in the gradient of the density of saccharose through method Glick with co-authors. Solutions, utilized for liberation/excretion in density gradient: 1) Versene-phosphate buffer (0.190 g of sodium salt EDTA, 2.86 g $Na_2HPO_4 \cdot 12 H_2O$ and 0.272 r $KH_2PO_4$ on 1 l of the distilled water); 2) the stock solution of the saccharose: 53.7 g of saccharose on 46.3 ml of Versene-phosphate buffer; 3) the working solutions of saccharose: a) 1.2 g of saccharose on 7.95 ml of stock solution, b) 1.65 g of saccharose on 6.55 ml of stock sclutiсn, c) 1.3 g of saccharose on 5.15 ml of stock solution. Each of the working solutions they finish to 10 ml by buffer and store at 4°C. The

preparation of the gradient: for days to experiment into centrifugal test tube by Pasteur pipette carefully laminate on 1 ml of each of the working solutions in the following crder: a, b, c, with the minimum mixing of phases. After 24 hours the gradient is finished to room temperature. Peritoneal suspension in volume 3-4 ml they carefully laminate to saccharose, and interphase between them they disturb by the light/lung circular motion of glass rod. Centrifuging is conducted during 10 minutes with 700-800 r/min on condition of the gradual growing of speed and smooth inhibition. After centrifuging there become well distinguished 3 layers of the cells: on the boundary of layer c and b - the dense whitish film of leukocytes, is below, in layer b - leukocytes and adipose cells; on the boundary of layer b and a - the tender diffusion greyish cloudlet of adipose cells.

Page 30.

This layer of cells they exhaust by syringe with long fine/thin needle. Adipose cells resuspend in the solution of hemocele, they wash clean from saccharose, repeatedly they centrifuge with 600 r/min during 10 minutes and breed into 1 ml of the solution of hemocele.

Direct/straight test of degranulation of adipose cells. To the slide, painted by 0.3o/o solution neutral red on absolute alcohol,

will be brought in to 0.05 ml of peritoneal suspension or suspension of adipose cells from the actively sensitized rats (to 9-16th day of sensitization). To this drop they add 0.05 ml of specific antigen. Mixture covers with cover glass whose edges are greased by vaseline, and they place into thermostat with 37°C. Reaction they read after 10-15 minutes: to 50 adipose cells, they compute the number of degranulated cells.

Indirect test of degranulation of adipose cells. To the painted neutral red microscope slide will be brought in 0.05 ml of the undiluted serum being investigated, which contains antibodies (immune serum of rabbit, reagin serum of man). To it they add 0.05 ml of the suspension of peritoneal or pure/clean adipose cells from the unsensitized rat and 0.05 ml of specific antigen (allergen). Mixture we cover with cover glass and place into thermostat in 10-15 minutes. As controls in this case, use: 1) a suspension and an antigen (allergen); 2) the suspension and the serum being investigated.

Passive sensitization of adipose cells. The small pieces of mesentery 2.5 x 2.5 cm in size/dimension of, obtained from intact rats and stretched to cover glass, incubate in immune rabbit serum for hour with 37°C. After incubation the preparations place into the solution of specific antigen (horse serum in breeding/culture/dilution of 1:10, the extract of pollen of plants in

breeding/culture/dilution of 1:10) in 10 minutes. Then preparations they record/fix, they stain with toluidine blue and they consist into balsam.

The expressed changes are noticeable in the adipose cells of shock organ/controls (light/lung of guinea pig, the mesentery and the peritoneal cells of rats, the liver of dog, bronchi of man). Through several minutes after the contact of adipose cells with specific antigen they begin somewhat to be increased in size/dimension, in peripheral regions appear vacuoles. The latter increase, "burst themselves" and issue their content in the extracellular medium. As a result of this vacuolization, adipose cell appears "bubbling". These changes it occurs approximately during minute.

The character/nature of the degranulation of adipose cells under the effect of specific antigen differs from their degranulation, caused by nonenzymatic liberator of histamine - by octylamine. In the latter case of change, they are developed in the loss of the sharp outlines of grains and diffusion of metachromatic substance. As a result the cell frequently becomes similar to the metachromatic macula in which sometimes are examine/scanned basophilic of granule. In last/latter stage this metachromatic macula loses even outlines, metachromasis weakens and cell begins to be lysed. Antigen causes only the partial loss by the cells of the grains whose outlines

remain sharp (Nelson et Al, 1968). Electron microscopy made it possible to differentiate two of basic types grains of the peritoneal cells of white rats (Blom, Haegermark, 1967). Ncrmal of granule are homogeneous and electronmicroscopic dense, they have sharp outlines, are surrounded by diaphragm/membrane. Those whc were modulated of large-size granule, possess less electrcn-microscope density, and in them becomes noticeable reticular subgranular grid/network.

Page 31.

Under anaphylaxis, the influence of substance 48/80 and of bee poison, sharply grow/increases the number of modulated grains, while with the application of decylamine and distilled water, they are unitary.

The question concerning that, is necessary for the release of histamine the output/yield of grains from adipose cells, is not finally solved. Hill (1957) is considered possible the release of histamine by the vacuolization of cell without the extrusion of grains. One way or another, the degranulation of adipose cells is accompanied by the release of them of the histamine and other biologically active materials.

Like other manifestations of anaphylaxis, the degranulation of

adipose cells and the release of them of biologically active materials depend on temperature. Optimum reaction reaches at temperature of 38°C. Increase in the temperature to 45°C or lowering to 20°C inhibits the anaphylactic reaction of adipose cells. The optimal pH value for the release of histamine is 7.5. The participation of enzymatic reactions in the process of the anaphylactic release of histamine from adipose cells was shown by the application/use of different inhibitors. Enzymatic process of the release of histamine is attained only in the presence of ions Ca.

The test of the degranulation of adipose cells recently is used for the detection of the allergic antibodies of man (L. I. Zelichenko, 1969).

Allergic reaction of leukocytes. The modelling of allergic reactions of the immediate type of man at cellular level encountered significant difficulties due to the absence of the adequate/approaching object for investigation in vitro. The systematic studies of recent years made it possible to develop the standard experimental model, consisting of the reproduction of the allergic reaction of the leukocytes of man, subjected to the action/effect of specific allergen.

Are possible two methods of the evaluation of this reaction as

the anaphylactic reaction of the adipose cells: on the release by the cells of histamine and with respect to a change in morphology of leukocytes. As in the case of the anaphylactic reaction of adipose cells, allergic reaction of the immediate type of leukocytes is explained by the compound of antibody with specific antigen on cellular surface or near it, as a result of which is release in extracellular liquid/fluid of the histamine and other biologically active materials. The majority of the investigations, made on the model of the allergic reaction of the leukocytes of man, is carried out with the utilization of pollen allergens.

For the execution of reaction, the leukocytes secrete from the blood of patient with the increased sensitivity to pollen allergen by centrifuging in the presence of dextran and anticoagulant (EDTA). Cells wash clean, they resuspend to the necessary volume and mix with specific allergen. In the case of the increased sensitivity to the pollen of ambrosia, was used as allergen the refined highly protein component (molecular weight 38000) (King et Al, 1964). The reacting system contains the leukocytes, suspended in NaCl-KCl-tris-buffer with 0.03o/o solution of serum albumin of man, the necessary cations about which will be said below, and the allergen. After the incubation at of 37°C cell, they deposit, and supernatant liquid they investigate to the content in it of histamine.

The high-sensitivity method of determining the histamine is spectrophotofluorometric method.

Factors, which affect the release of histamine from the leukocytes of man. To the optimum conditions of the reproduction of reaction correspond to pH 7.4 and the ionic force of solution, equal to 0.15. The addition of the ions of magnesium ($10^{-3}$-$10^{-2}$ moles) raises to a higher power the anaphylactic output/yield of the histamine which reaches 80-100o/o of entire cellular reserve of this amine.

Page 32.

The output/yield of histamine even in the final stages of reaction is complex diffusion through the roughly damaged cellular diaphragm/membrane. This process requires the preservation/retention/maintaining of the metabclic activity of cell. The study of the effect of blockers of enzymatic reactions to the anaphylactic output/yield of histamine confirms this position. Preliminary treatment of leukocytes with the inhibitor of esterase activity diisopropylfluorophosphate ($5 \cdot 10^{-3}$) completely inhibits the output/yield of histamine, without affecting the immunological stage of reaction (Osler et Al, 1965).

Research on the effect of concentration allergen on the degree of the output/yield of histamine from leukocytes, carried out on the model of the increased sensitivity to ambrosia, showed that the concentrations of the purified protein allergen, calling the minimum and maximum release of histamine, considerably vary depending on the utilization of cells on different people. These oscillation/vibrations reach hundredfold. So, threshold concentrations vary from $10^{-5}$ to $10^{-3}$ μg of protein of allergen to 1 ml, and maximum - from $10^{-4}$ to $10^{-2}$ μg. The excess of antigen inhibits the anaphylactic release of histamine. In the majority of cases of the reproduction of the anaphylactic reaction of leukocytes, actually is free/released entire cellular histamine. This testifies to implication in the allergic reaction not only of the basophils, but also other leukocytes.

Research on the temporary course of the reaction of the release of histamine showed that maximum the reaction reaches (with the maximum concentration of allergen) on the 20-40th minute after the addition of allergen.

The fact of the fixation of antibodies on the surface of leukocytes was establish/installed by autoradiography with the utilization of protein, mark/tagged $I^{131}$ - the allergen of ambrosia (Osler et Al, 1965). The joining of marked allergen by these cells

was considerably inhibited by preliminary addition to the cells of the excess of the unmarked allergen.

Passive sensitization of the leukocytes of man in vitro. The leukocytes of man, obtained from donor, who did not suffer allergic diseases, can be sensitized passive reagin serum (Osler et al, 1965). This procedure consists in the holding of the isolated/insulated leukocytes in the blood serum, which contains reagin, under the physiological conditions pH and the ionic force of solution. After the thorough washing of serum, the addition of allergen to leukocytes causes the release of them of histamine. General conditions and the laws governing the passive sensitization of leukocytes (effect of the temperature, ionic medium, duration of sensitization) correspond to the laws governing the passive sensitization of other cellular structures and tissues (Osler et al, 1965), by in detail described in the example of passive sensitization in the example of the passive sensitization of smooth-muscle organ/controls.

There are considerable oscillation/vibrations in capability for sensitization of different donors's leukocytes. So, during testing of 84 donors's leukocytes only in 45 was reveal/detected the capability of leukocytes for sensitization and the subsequent anaphylactic release of histamine (Patterson, 1969).

Model of allergic alteration of the leukocytes of man. In V. B. Gervaziyevoy's our laboratory (1968) is developed the test of the allergic alteration of the leukocytes of man with the application/use of a method of luminescence microscopy with the "in vivo" stain of leukocytes by acridine orange. This method is more sensitive and makes it possible to judge not only the morpholcgical manifestations of the alteration of leukocytes, but also about the functional state of cells by a quantity of absorbed dye/pigment.

Because of relative simplicity of reproduction, this model of cellular allergic reaction is used for the diagnostic target/purposes of the determination of allergic antibodies with pollinoses. Method was tested in the example of the increased sensitivity of sick with pollinosis to pollen gramineous grasses (timothy grass, the fescue, hedgehog polythalamous), ragweed and some trees.

Page 33.

The forward reaction of the alteration of leukocytes. In 2 silicone Widal test tubes are compose/collected 0.45 ml of the blood of patient with anticoagulant. As anticcagulant is used 1.5o/o solution of disodium salt EDTA in physiological solution. Then into test tube they add 0.05 ml of specific allergen in breeding/culture/dilution of 1:10 in the Simms solution to which in

patient is reveal/detected the increased sensitivity. In another test tube they add the same quantity of nonspecific allergen (control). Mixture in test tubes carefully is mixed and they leave at room temperature for 1 hour. Then content of test tubes is again carefully mixed and added into each test tube on 0.05 ml of the salt solution of acridine orange in breeding/culture/dilution of 1: 20000. Exhibition/exposure with the color/paint of 5 minutes. Basic solution acridine orange is prepared on the distilled water in breeding/culture/dilution of 1: 1000 and retain in darkness with 5°C during several months. Working solution is prepared from basic in the Simms solution in final breeding/culture/dilution of 1: 20000 and is used not longer than 2 weeks; the pH of salt solution of acridine orange is not less than 6.3.

After stain/staining the drop of mixture will be brought in to microscope slide, they cover/coat with cover riding-crop and the obtained vital preparation they investigate in flourescence microscope (MP-2). In the first of 3-5 minutes of cell, they are arrange/located on the strength of severity in one plane; their noticeable motion is stopped and thereby is virtually eliminated the double calculation of the same cell. In several fields of view (on 8-10 cells in each) they compute 100 granulocytes and calculate the percentage of the deformed leukocytes.

Passive reaction of the alteration of leukocytes. In 0.2 ml of sound/healthy donor's one-piece/entire blood I (O) the groups of blood or 0.2 ml of the washed clean cellular suspension add 0.2 ml of reagin blood serum of the patient with pollinosis. Then to the obtained mixture add 0.05 ml of specific allergen. To each experiment correspond controls to allergen and reagin sera. Mixture they incubate at room temperature for hour, is prepared the vital fluorochromized preparation and they compute in flourescence microscope the percentage of the modulated leukocytes.

The neutrophils, painted by acridine orange, have dull green luminescence of cytoplasm, almost invisible due to the abundance of ruby-red grains in cytoplasm. Nuclei are light green with sharp, smooth outlines. Eosinophils are characterized by from neutrophils the denser graining in cytoplasm and the presence of most frequently two-fragment nucleus. Basophils contain the polymorphic bright green nucleus, which fills almost entire cell, and large clear red nonabundant graining in cytoplasm. The nuclei of lymphocytes have emerald -green luminescence, cytoplasm dull green with unitary red grains.

A similar luminescence of leukocytes is explained by the capacity of the acridine of orange differentiated to interact with nucleic acids. So, RNK of cytoplasmic grains binds a large quantity

of dye/pigment and gives the complexes of a ruby-red luminescence. DNK binds the dye to a lesser degree and gives the bright green luminescence. The weak luminescence of cytoplasm is caused by the facts that the extranuclear RNK is found in bound state with other components of cell and does not have available bonds for reaction with dye/pigment.

Page 34.

The sensitized leukocytes of the patients under the effect of specific allergen are subjected to the distinctly expressed morphological alteration, moreover the visible alteration is revealed exclusively of granulocytes. According to the degree of manifestation morphological changes with the allergic alteration of leukocytes can be subdivided into 4 stages. Stage I is leukocytes of usual globular form, externally not differing from the nonmodulated cells, but characterized by an increase in the luminous intensity of cellular components. Stage II- leukocytes with the uneven "torn" edges and the beginning evacuation/rarefaction of cytoplasm on periphery. Stage III is leukocytes with the explicit defect of cellular boundaries, with the phenomena of degranulation, vacuolization and the swelling of cytoplasm. Stage by the swelling of cytcplasm. Stage IV is leukocytes from homogenization and by the significant disappearance of cytoplasm, by coarsening chromatin figure of nucleus. For the

reaction of stage II-IV characteristically gradual lowering in the luminous intensity of cellular components.

In the special study of the mechanism of the allergic alteration of leukocytes (V. B. Gervaziyeva, 1968) it is shown, that the development of this process depends on the presence of the circulating antibodies which according to their thermolabile properties and bond with the determined types of immunoglobulins can be referred to reagin.

Allergic reaction of basophilic leukocytes. This reaction occupies special place in the modelling of cellular allergic reactions, since precisely basophils contain about 50o/o of entire cellular histamine of the blood. In 1961 Shelley proposed to use basophilic leukocytes as test object for research on immediate type allergic reactions. Since in the peripheral blood of man basophils are only 0.35-1o/o of all leukocytes, was developed indirect test on the basophils of rabbit. These animals have the content of basophils in the blood sufficiently high (4-8o/o). Indirect test is based on the reaction of basophils during the reaction of specific allergen with the antibodies of the blood serum of patient. The indicated reaction received sufficiently wide acceptance in the diagnosis of allergic diseases by the name of the indirect test of Shelley. With the aid of this test it was possible to reveal/detect allergic

antibodies in the blood of the patients with the increased sensitivity to different pollen allergens and medicinal substances. The theoretical value of this reaction lies in the fact that it is the model of anaphylaxis in miniature.

Research on the mechanism of the allergic reaction of basophils, carried out in our laboratory (A. D. Adc et al., 1970; A. A. Pol'ner, 1968, and other), led to the establishment of the new data which, possibly, are important for the explanation of the mechanism of anaphylaxis of other cellular cell/elements. The reaction of basophilic leukocytes to specific allergen is developed morphologically in the degranulation of these cells, and the release of the histamine or other biologically active materials explains by the disturbance of the completeness of cell and by the output/yield from it of grains. The observation of the course of an entire reaction of basophils, made in our laboratory with the utilization of micromotion picture filming, showed that this reaction is developed in following sequence. Through 10-15 minutes after action on the basophils of specific allergen and the corresponding to it antibodies, occurs the "activation" of cell, designated "granulokinesis". Is observed the swelling of grains, the sharp strengthening of their vibration, formation pseudopodium and the displacement/movement in them of grains. However, cell in this case does not disintegrate and granule do not fall outside it limit. Then,

as a rule, after 30 minutes, is observed the reduction of the usual form of cell. Granule from pseudopodium they return to the depth of cytoplasm, pseudopodia they disappear, the vibration of grains becomes less intensive and it gradually makes unnoticeable. With the usual of experiment on the Shelley method the calculation of cells they begin to conduct with the torque/moment, cooresponding to the height of morphological changes (after 10-15 minutes from the beginning of reaction), designated by "degranulation". Thus, then which was accepted as the degranulation of basophils, in actuality it is only one of the stages of the reaction of basophils in de the activation of their ameboid motion, "granulokinesis", which is completed by the state of relative rest of cell. The described fundamental fact attests to the fact that the release of biologically active materials from cell occurs, obviously, without the destruction of its diaphragm/membrane and most likely in the period of the "activation" of its state.

For the reproduction of reaction, are used the blood serum of the patients with the increased sensitivity to pollen allergens. The leukocytes of rabbit obtain by centrifuging heparinized blood with 3000 r/min during 5 minutes in fine/thin glass test tubes. In the experiment it is expedient to use not the greatest concentrations of allergen and antibodies, but their optimal relationship/ratios, since depending on the titer of the allergic antibodies, detected by

reaction, the optimal concentrations of allergen and serum, which contains antibodies, for each patient are individual. To the microscope slide, pre-painted by 0.3o/o alcoholic solution neutral red, will be brought in the identical volumes (cn 0.005 ml) of leucocyte suspension, of the serum being investigated and allergen. Carefully they mix by their edge of cover glass. Mixture covers with cover glass whose edges are greased by vaseline. Preparation is placed into thermostat with 37°C in 15 minutes. In parallel are placed controls with allergen and serum. They ccmpute 40 basophils. Reaction is considered positive, if a quantity of changed basophils in experiment to 10o/o is more than in control. The dynamics of the process of morphological changes in the basophilic leukocytes can be photographed by time-lapse method to mction picture film.

Material about the modelling of allergic reactions makes it possible tc make some generalities.

The experiment of research on the mechanism of allergic reactions shows that the separate fundamental sides of the pathogenesis of the allergic diseases of man, at basis of which lie/rests immediate type increased sensitivity, can be sufficiently fully modeled in animals at systemic, organ, tissue and cellular level. The aforesaid entirely pertains to modelling, the immunological, pathochemical and pathophysiological stages of

allergic reactions.

However, the specific character of the course of allergic reactions in man frequently limits the possibility of utilization for modelling and the explanations of the mechanism of these reactions of the regularities which are obtained on the model of the allergic states of animals. In connection with this for the modelling of allergic reactions, is indisputably valuable the development of models on the isolated/insulated organ/controls, tissues and the cellular structures of man.

This direction successfully is developed recently, which is successfully demonstrated in the example of the creation of the models of the allergic reactions of the isolated/insulated smocth muscle organ/controls and regular cell/elements of the blood of man. Although the question concerning the possibility of the modelling of allergic reactions on the tissues of man it is possible to consider mainly solved, all the same development of similar investigations will become attained only under the condition of developing the problem of conservation and creation of the reserves of the isolated/insulated tissues of man. The development of these models and their utilization in experimental and clinical allergology will make it possible to faster solve the problem of control of the allergic reactions of man at the different stages of their development.

DISTRIBUTION LIST

DISTRIBUTION DIRECT TO RECIPIENT

| ORGANIZATION | | MICROFICHE |
|---|---|---|
| A205 | DMATC | 1 |
| A210 | DMAAC | 2 |
| B344 | DIA/RDS-3C | 8 |
| C043 | USAMIIA | 1 |
| C509 | BALLISTIC RES LABS | 1 |
| C510 | AIR MOBILITY R&D LAB/FIO | 1 |
| C513 | PICATINNY ARSENAL | 1 |
| C535 | AVIATION SYS COMD | 1 |
| C591 | FSTC | 5 |
| C619 | MIA REDSTONE | 1 |
| D008 | NISC | 1 |
| H300 | USAICE (USAREUR) | 1 |
| P005 | ERDA | 1 |
| P055 | CIA/CRS/ADD/SD | 1 |
| NAVORDSTA (50L) | | 1 |
| NASA/KSI | | 1 |
| AFIT/LD | | 1 |

| ORGANIZATION | | MICROFICHE |
|---|---|---|
| E053 | AF/INAKA | 1 |
| E017 | AF/ RDXTR-W | 1 |
| E404 | AEDC | 1 |
| E408 | AFWL | 1 |
| E410 | ADTC | 1 |
| E413 | ESD | 2 |
| | FTD | |
| | CCN | 1 |
| | ETID | 3 |
| | NIA/PHS | 1 |
| | NICD | 5 |