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PROBLEMS RELATED TO IMMUNIZATION WITH LIQUID VACCINE AEROSOLS

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The use of liquid vaccine aerosols for immunization has shown that this method has some advantages over parenteral administration, in particular there are no significant postvaccinal reactions, they are highly effective, and they make it possible to immunize large groups of people simultaneously, etc. However, in practice, this method has not yet found wide application and this is apparently related first of all to the difficulty in determining the actual vaccination dosage reaching the respiratory organs, especially when the aerosol is in a polydispersed state. In the area of aerosol immunization of visible scientific interest are the following questions: determination of the site of primary application of the antigen, conditions for immunization against each infection individually in accordance with pathogenesis and natural mechanism of infection; nature of immunological and pathomorphological changes primarily in immunized lungs as well as reactogenicity and safety of aerosols as related to various vaccines. Of course, in order to solve these problems the existing aerosol immunization techniques must be approved and an improved one should be developed as well as monitoring and measuring equipment without which in essence progress is impossible with respect to solving this complex aerobiological problem.

Requirements for the Use of Vaccine Aerosols for Immunization

Precipitation of aerosol particles in the human respiratory tract. Many articles have been published on the distribution and precipitation of aerosols in the respiratory system (N.A. Fuks; I.I. Yelkin and S.N. Eydel'shteyn, 1955; B.V. Deryagin; Brown et al; Sonkin; Mitchel; Dauterbande and Walkenhorst; Casarett; Hatch).

Many studies have also offered an idea as to the influence of the

aerosol particle size on retention in the respiratory tract. The article by Van Wijk et al clearly shows that total retention in the lungs constitutes 96% with particles 5 microns in diameter, 42% when they are 0.5 microns in diamter, and 21% with an 0.2 micron diameter. There must be as many one-micron or smaller particles as possible for penetration into the deep parts of the lungs down to the alveolar spaces. Findeisen estimated that only 2.6% of particles 1-2 microns in diameter are exhaled, whereas retention of particles 0.2-0.6 microns constitutes 66%, and retention is again higher with respect to even smaller particles; a second retention wave is also confirmed by Landahl and Tracewell (1951, 1952).

To sum up the theoretical and experimental data regarding the influence of particle size on their retention, Brown et al noted that optimum retention occurs with 5 micron particles. This recedes to a minimum (25%) with particles 0.25 microns in diameter, then rises again with submicron particles. Retention in the nose is observed with particles over 5 microns in diameter of practically 100%, which decreases with decr decrease in size attaining zero with one micron particles. The depth of aerosol penetration increases with decrease in particle size. Particles of about one micron which are not retained in the upper tract are totally retained in the alveoli. The precipitation percentage increases for particles under one micron. Thus there is an optimum particle size (about one micron) with the highest probability of settling in the alveoli. There is approximately the same probability of retention in the lungs for smaller particles (0.25 micron) and those that are one micron in size. With respect to submicron particles, with decrease in their size there is increased ability for retention and it approximates the maximum in accordance with the quantity of air exchanged in one respiration that reaches the alveoli. The correlation between particle size, penetration and precipitation changes with fluctuations of respiratory volume, i.e. retention increases with decrease in respiration rate.

Hatch and Hemeon (1961, 1948) demonstrated that the percentage of inhaled and precipiated particles in the pulmonary spaces depends on the sum of three magnitudes: fractions of particles that have avoided settling in the upper respiratory tract, percentage of inhaled air penetrating into the lobe, and intensity of precipitation in the lungs. It is noted in their articles that the highest probability of precipitation of inhaled particles in lung tissue prevails with particles 1-2 microns in diameter as well as with submicron (0.2 micron) particles. Penetration and settling in the pulmonary lobes decrease for particles over 1-2 microns in diameter simply because a large quantity of particles settles in the upper respiratory tract. In essence the probability of particles over 10 microns in diameter settling in the lobes is nil. With particles smaller than 1-2 microns in diameter the intensity of precipitation in lung tissue drops, however it again rises with particles under 0.25 micron in diameter since the force of precipitation by diffusion is increased with decrease in particle size (Figure 1).

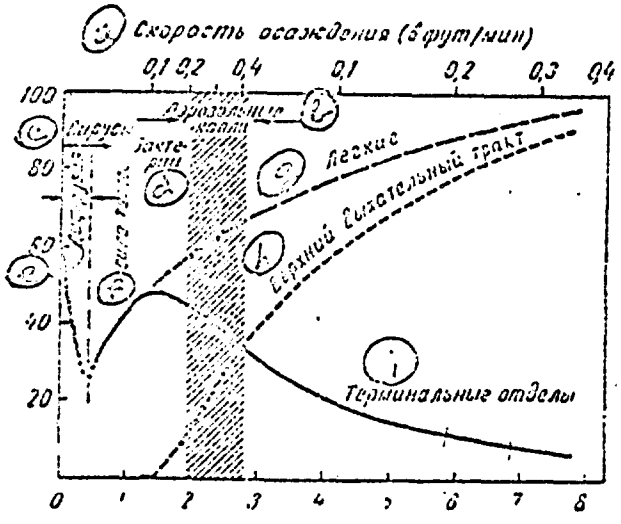

Figure 1. Degree of retention of different sized aerosol particles in the upper and lower respiratory tract (according to Hatch, 1961)

Abscissa -- diameter of aerosol particles (inmmicrons)
Ordinate -- inhaled particles settling in different parts of the lungs (%)

Legend:
a) precipitation rate (in feet/minute)
b) aerosol drops
c) viruses
d) bacteria
e) diffusion
f) gravity
g) lungs
h) upper respiratory tract
i) terminal segments

Data on depth of penetration of aerosol particles and effectiveness of their settling in different parts of the human respiratory system (Findeisen) are submitted in the Table. N.A. Fuks considers these data to be somewhat idealized, however they reflect accurately enough the general tendency of aerosol precipitation.

On the basis of Findeisen's data and their own findings, Kadlec et al submitted the results of aerosol particle retention in respiratory organs in the form of a graph (Figure 2) in which we see that prevalent settling in specific parts of the respiratory tract is related to particle size. As we know the latter is of fundamental importance in aerosol immunization and medical practice (inhalation).

The physical condition of the sol also influences penetration of aerosols into different segments of the lung. Thus, Landahl and Tracewell indicated that the diameter of particles penetrating through the nose (50%) ranged from 4.5-6.7 microns for nonhygroscopic materials (methylene blue, bismuth subcarbonate), and 2.5 microns for hygroscopic materials (glycerin).

Effectiveness of aerosol precipitation in different parts of the human respiratory system

(a) Отдел дыхательного тракта	(b) Эффективность осаждения (в %) при радиусе частиц (в мк)						
	0,03	0,1	0,3	1	3	10	30
(c) Трахея	0,16	0,08	0,03	0,10	0,8	7,8	67
(d) Бронхи главные	0,61	0,21	0,10	0,07	0,11	0,7	—
(e) первого порядка	0,28	0,13	0,07	0,07	0,04	2,5	—
(f) второго порядка	0,55	0,26	0,15	0,14	0,85	5,...	—
(g) третьего порядка	1,03	0,51	0,29	0,35	2,00	5,3	—
(h) Бронхиолы							
(i) концевые	6,10	3,10	2,00	4,00	25,00	10,2	—
(j) респираторные	6,30	3,20	2,00	3,70	16,00	—	—
(k) Альвеолярные ходы	37,20	19,10	15,80	40,30	36,60	—	—
(l) Альвеолы	14,10	8,00	12,70	41,60	—	—	—
(m) Выводится из легких	34,00	65,00	65,80	2,60	—	—	—

Legend:

a) segment of respiratory tract
b) effectiveness of precipitation (%) at a particle radius (in microns) of:
c) trachea
d) primary bronchi
e) primary
f) secondary
g) tertiary
h) bronchioles
i) terminal
j) respiratory
k) alveolar tract
l) alveoli
m) excreted from the lungs

However it is still not enough to know the size of the aerosol in order to determine where it settles in the lungs. Here other factors are also involved: cough impulses, profusion of sputum, condition of mucosa of the respiratory tract especially of the ciliated epithelium (Wright, 1961; Bang), degree of phagocytosis of foreign particles (Shoskes et al) as well as configuration of particles (Bedford and Warner), electric charge (E.I. Raudam et al), rate of air movement, and other physical factors (B.V. Deryagin; Thomas).

The degree of particle retention is also related to respiration rate. Particles 0.1 to 0.7 micron in size at a respiration rate of 32, 10 and 3 per minute settle in the lungs in 37, 60 and 90% respectively (Dauterbande and Walkenhorst). An increase in respiratory exchange accelerates particle precipitation in the alveolar spaces. Therefore settling of particles in the deep-lying segments will probably be more effective during breath holding.

Thus, for more complete retention in the human lungs, the aerosol particles should be 1 to 3 microns in diameter, which is possible when using liquid fractions; as yet it has not been possible to obtain dry aerosols (vaccines) of such dispersion. In addition, as a result of

rehydration, dry particles in an air system usually enlarge whereas fluid ones diminish (Zentner).

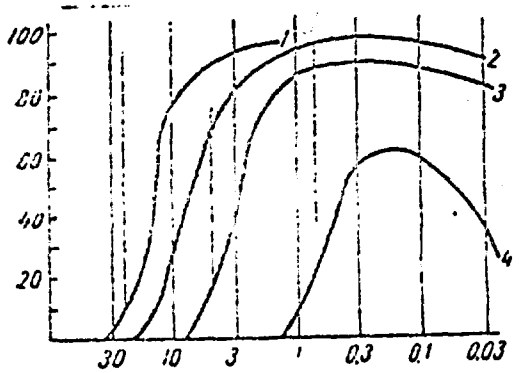

Figure 2. Retention of aerosol particles in respiratory organs (according to Kadlec et al, 1959)

Abscissa — diameter of aerosol particles (in microns)
Ordinate — retention of particles (%)

Legend: 1) trachea
2) bronchi
3) bronchioles
4) alveoles

The dotted lines show optimum retention of particles of each size.

The fate of aerosols in the respiratory system. Work in this direction was performed using different agents: dyes (A.K. Yyentes, 1962; Lyons et al, 1944; Elson), protein solutions (Fox; Drinker and Warren; Courtice and Phipps; Lamanna), pharmacological preparations (Dauterbande, Lovejoy), labelled antigens (Goldberg, 1950; Goldberg and Leif; Logan et al), isotopes (M.Ya. Mayzelis; Yu.I. Moskalev et al, 1962; Frieberg; Bair and McClanahan; Berke and Pasqua), as well as live and killed bacteria (B.A. Boyarinova; M.P. Pokrovskaya et al; Ames and Nungester; Guarneri and Endriga; Van de Loo; White et al, and others).

To summarize the data on the mechanism of absorption and fate of aerosol particles in the respiratory tract and lungs we can state the following.

1. Absorptivity of the respiratory tract mucosa increases gradually from the nasal cavity down to the lungs and is highest in the alveolar region. This is related to the anatomical and physiological distinctions of the lungs rich in blood and lymphatic vessels as well as the colossal size of the alveolar surface which, in man for example, equals 50 square meters (according to some data — 120 square meters), and this is several tens of times greater than the body surface (V.N. Zhedenov).

2. As a result of absorption, inhaled substances penetrate rather rapidly into the general lymph and blood stream of the organism. According

to G.F. Ivanov (1940) several minutes after inhalation the indicator (potassium ferrocyanide) penetrates into the closest lymph nodes. Drinker and Hardenbergh found dye in the blood within the first few minutes, Dragsted and Schwarz detected penicillin in the urine within 10-15 minutes after inhalation. However solutions containing protein are absorbed considerably slower (Drinker and Warren; Fox).

Experiments using aerosols of pharmacological preparations are illustrative in this aspect (Dauterbande et al, 1957, 1961): the effect is observed within a few minutes and persists for a longer time than following subcutaneous administration. When using radioactive aerosols about one micron in size (I.A. Frolova; K.G. Scott et al; Jech) radioactivity was established in rats' blood as soon as ten minutes after inhalation.

Distribution in the organism of radioactive agent aerosols after they have been inhaled has been studied in detail by many researchers with consideration of dispersion, form of compound administered, physicochemical properties, etc (N.D. Sagaydak; Bair and McClanahan; Pickroth et al; Kajland et al; LaBelle et al). It was shown that soluble compounds are absorbed considerably faster in the lower segments of the respiratory tract and then disperse in the organism through the lymph and blood stream as well as through phagocytosis.

3. What then is the mechanism of penetration into the lymphatic and blood stream of vaccine aerosol particles that have reached the deep segments of the lungs? Let us submit the data from some studies.

Van deLoo who immunized rabbits with killed paratyphoid vaccine (aerosol size: 2 microns) demonstrated that the cells of the alveolar epithelium phagocytize antigen which then gets into the lumen of alveoli and bronchioles. If the phagocytic cell dies off in the alveolus the released antigenically active components penetrate into the alveolar capillaries and then into the general blood stream, and partially into the lymphatic stream. If the cells of the alveolar epithelium are excreted into the bronchiolar lumen, the released antigen reaches the mucosa of the bronchioles and along the intercellular lymphatic fissures goes on to the submucosal layer where it elicits proliferation of plasmatic cells. Some of the alveolar epithelial cells which phagocytized antigen are excreted outside, which was also demonstrated by Hilding (1963), Casarett and Milley (1964).

Other hypotheses are also expounded as to the means of migration of antigen from the lung into the general lymphatic and blood stream. Brieger and Ferin et al believe that phagocytes travel from the blood stream into the pulmonary air space where they pick up the foreign material then return into the interstitial space, from which they reach the lymph stream. Green and Kass related the exist of antigen from pulmonary epithelium to alveolar macrophages. A.P. Gindin et al point to the large

quantity of lymphocytes rich in RNA in the sinuses of lymph nodes in aerosol immunization, from which they enter the blood; it is also possible that there is direct penetration into the lymphatic system of the lungs by mechanical suspensions and bacteria through absorption from the alveoli and bronchioles (D.A. Zhdanov).

As seen from the data submitted, the process of migration from the lungs of bacterial preparations that have reached the alveolar spaces is rather diverse and complex, but apparently the main factor is phagocytosis.

Dosimetry in aerosol immunization. While it is possible to determine rather accurately the quantity of administered vaccine when the parenteral method is used, with aerosol administration it is rather difficult to measure the dosage. This is related to the purely physical properties of aerosols (sedimentation, coagulation, changes in concentration, dispersion, etc) continuously observed in the aerosol cloud, as well as to the distinctions of aspiration, primary settling in respiratory organs, respiratory ventilation, etc. In other words, in order to determine the inhaled dose it is necessary to obtain experimentally, using instrumental methods, a number of data influencing the magnitude of the inhalation dosage.

In a simplified form, the equation for calculation of inhaled dosage (D) has the following appearance:

$$D = C \cdot V \cdot P \cdot t \cdot R,$$

where C is the concentration by weight of sprayed agent (in grams per milliliter), V is the animal's volume of pulmonary ventilation (in milliliters per gram of body weight), P is the animal's weight (in grams), t is the time of contact with the aerosol (in minutes), R is the percentile retention of inhaled aerosol in the respiratory tract.

Each of these parameters can change considerable depending on several conditions (type of atomiziers and atomized agents, fractional composition of aerosol, temperature, humidity, weight of the experimental animal, etc). Nevertheless it is possible to determine these parameters experimentally and to calculate the final sought value (inhalation dosage) more or less close to the actual one.

The concentration (C) is calculated by multiplying the number of aerosol particles per milliliter of air by the weight of one aerosol particle (the radius is known). For this purpose an aerosol concentration counter can be used, in particular the domestic instruments -- ultramicroscope with laminar (B.V. Deryagin and G.Ya. Vlasenko)* or intermittent flow of air (Ye.A. Vigdorchik; G.S. Berezyuk). Using them it is

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*Continuous operation ultramicroscope, VDK_4.

possible to determine as well the dispersion of aerosols. In order to obtain more precise data on dispersion of the particles their fractional distribution in time must be examined. The latter is particularly important when using polydispersed aerosols.

Most researchers obtain the values of pulmonary ventilation volume (V) from the data obtained by Guyton (1947a, b) and Rosebury on the basis of complex research methods.

The animals' weight (P) and time of contact with the aerosols (t) can be readily determined through experiments. At the first approximation the product of the above parameters (C·V·P·t) furnishes the magnitude of the inhaled dose (D) if we assume that the entire dosage in the inhaled air was retained in the respiratory organs.

However, it follows from the experiments with radioactive aerosols that the estimated inhalation dosage according to this equation is somewhat higher than in actuality (Palm; Mitchel). Thus, for example, according to the report of Goldberg and Leif.(1950) retention of inhaled aerosol with a particle radius of about one micron in mouse organs constitutes no more than 27-30%; according to Schechmeister (1950), Harper and Hood (1962) -- about 50%, and in man (Punte et al, 1963) up to 50-75% (depending on the nature of respiration).

To summarize the foregoing it may be stated that determination of inhaled dosage of aerosols by existing estimation methods permits obtaining values relatively close to the actual ones.

Aerosol Immunization Against Viral and Bacterial Infections

In 1920 a method was proposed of creating passive immunity by intratracheal administration of immune sera. It was believed that not only does this method cause production of strong immediate immunity but also that it helps avoid the danger of anaphylactic shock in the case of high sensitivity to intravenous administration of the serum.

The results of subsequent studies performed in order to determine the effectiveness of intratracheal and intranasal administration of immune sera are rather contradictory (Zellat and Henle; Krueger et al; Lyons). However inhalation of atomized immune serum or globulin fraction is unquestinably effective prophylactically and therapeutically (A.V. Nechayev et al; O.M. Chalkina et al; N.N. Orlova; A.A. Smorodintsev and O.I. Shishkina; A.A. Smorodintsev et al; A.N. Slepushkin; K.M. Sinyak et al; A.S. Shadrin; Hopps and Moulton), especially by means of aerosols. By now there is a rather large number of infectious diseases in which aerosol immunization is being used with success.

Influenza. Ye.T. Korobkova and N.S. Salun (1934) tested on volunteers the aerosol method of immunization using inhalation apparatus, and

observed a positive effect. O.M. Chalkina (1938) also indicates the absence of reactogenicity following inhalation immunization against influenza: she immunized 74 people three times, 59 -- twice, and 43 -- once. None presented any local or general reactions; a five to 25-fold increase in virus-neutralizing antibodies in the blood was observed.

However, Francis and Pearson (1944) who used finely divided live influenza virus aerosols concluded that the method was reactogenic. In this connection of interest are the following data of A.A. Smorodintsev (1950). Administration of attenuated live virus into the lower segments of the respiratory tract elicited in some of the inoculated individuals some clinical symptoms of influenza infection which were all the stronger the lower the antibody titer before infection. In response to single or repeated inhalation of attenuated virus there was an increment of antibody titer in the blood, and the lower the titer before infection the greater was this increment. In view of these data the conclusion made by Francis and Pearson becomes understandable: reactogenicity of aerosol immunization against influenza for individuals who failed to present antibodies prior to immunization. The comparative study made by O. I. Shishkina (1938) of effectiveness of various vaccination methods in experiments on mice indicated the advantages of the aerosol method.

Unfortunately these early works failed to include information about the particle size of the aerosol, estimation of inhaled dose of vaccine, etc., so that we cannot make a comprehensive evaluation of their significance in immunization against influenza.

Measles. There are very few studies on the use of the inhalation method of immunization against measles. Thus, Japanese researchers (Okuna; Okuna et al; Minamitani et al) demonstrated that after subcutaneous inoculation (triple is best) with inactivated antimeasles vaccine and reimmunization by the inhalation method with live vaccine (Siguyama strain) children developed intensive immunity as good as post-infection immunity. Reactions were essentially absent. High titers of complement fixing and virus neutralizing antibodies were found. Inhalation and intranasal vaccination of children who had not been previously inoculated and whose blood did not contain the appropriate antibodies induced a reaction with clinical manifestations of typical vaccinal measles (Lee). Kress et all arrived at similar conclusions with regard to the inhalation method of immunizing against measles. These studies covering an adequate number of cases (from 50 to 100 or more) still fail to answer the main questions arising with aerosol immunization: what is the estimated inhalation dosage, depth of precipitation in the lungs, fate of the vaccine virus in the lungs, etc.

Ornithosis. In view of the distinctions of the pathogenesis of this infection, the site of primary localization of the pathogen of which are the lungs while the cells of respiratory bronchioles and alveolar epithelium are the most sensitivty (I.I. Terskikh et al, 1961; I.I. Terskikh; McCavran et al), we tried aerosol immunization to create local

immunity of the lungs or resistance of sensitive cells to the ornithosis virus. The results of experiments with aerosol immunization of animals with killed anti-ornithosis tissue vaccine are indicative of the efficacy of the method when aerosol atomizers and generators are used which permit creation of a high concentration of fine-particle fractions. Thus the logarithm of the resistance index in albino mice vaccinated once attained 2.5-3 or more with a counted concentration of particles of $9.6 \cdot 10^5$ to $9.8 \cdot 10^5$ per cubic centimeter of air (vaccine content by weight: 1.2-1.3 milligrams per liter). The efficacy of aerosol immunization increases when it is repeated in 7-10 days. A comparison of the effectiveness of vaccination in relatively comparable doses by aerosol, intranasal and intraperitoneal methods revealed the superiority of aerosols. Experiments on monkeys and pathomorphological examination of the animals' organs revealed the safety of aerosol immunization (I.I. Terskikh et al, 1961, 1964, 1965; A.I. Danilov et al, 1964, 1965, 1966).

Tick-borne encephalitis, encephalomyelitis. The main studies on aerosol immunization against tick-borne encephalitis* were performed at the Institute of Virology imeni D.I. Ivanovskiy, USSR Academy of Medical Sciences by the authors of the present article in collaboration with the staff of the laboratory of comparative virology (I.I. Terskikh et al, 1965; A.I. Danilov et al, 1965; A.I. Danilov et al, 1966). In experiments on monkeys we studied immunization with mixed vaccine against ornithosis and tick-borne encephalitis and noted the lack of competition between antigens with high immunogenic activity of the vaccine and resistance of animals (I.I. Terskikh et al, 1964).

The studies on aerosol immunization against Venezuela equine encephalitis were very thorough and complete (Kuehne and Gochenour; Hoarn; Woodward and Tigertt). The aerosol immunization study was conducted with the use of live vaccine. Animals (mice, guinea pigs and Macacus, Mulata monkeys) inhaled various doses of vaccine. There were no pathological reactions, and x-ray failed to reveal lung lesions.

In subsequent works, Kuehne et al, Sawyer et al furnished more precise determination of live vaccine dosage against Venezuelan equine encephalomyelitis (VEE) with aerosol immunization with monitoring by serological tests and testing of intensity of immunity following infection with virulent strains.

Of great interest is the last work. It submits the results of mixed (combined) aerosol immunization with live tularemia vaccine and live VEE vaccine. In 80% of the cases the aerosol particle size did not exceed two microns in diameter. Monkeys infected subcutaneously by

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*The vaccine was prepared at the Laboratory of Comparative Virology (headed by Professor A.K. Shubladze), Institute of Virology imeni D.I. Ivanovskiy, USSR Academy of Medical Sciences.

administration of 1000 virulent tularemia pasteurella five months after aerosol vaccination presented an adequate intensity of immunity. The authors observe that inhalation of mixed vaccine aerosols may serve as reliable prophylaxis against tularemia and VEE.

Plague (of fowl, pigs). A considerable number of Soviet studies has been published in the last two years showing the wide possibilities of using the method of aerosol immunization with liquid vaccines in veterinary practice for the purpose of preventing infectious diseases (V.I. Burtsev et al, 1964a, b, c; V.V. Chumyshev and V.I. Burtsev; I.I. Kulesko et al; O.V. Krivonosova et al, 1965a, b). The possibility of using production buildings (pig sties, aviaries, etc), the simplicity of the method and equipment, economic considerations favor the wide use of aerosol immunization in veterinarian practice.

Intestinal infections. Animals and humans exposed briefly to a cloud ["fog"] containing typhoid or dysentery vaccine developed antibodies in their blood without any adverse reactions. A comparison of the inhalation and enteral vaccination methods revealed the superiority of the former (Ye.I. Demikhovskiy et al).

Later experiments on respiratory immunization with liquid vaccines against intestinal infections were performed only on animals. Thus, the efficacy of inhalation vaccination with killed corpuscular Gertner vaccine, especially using a fine particle aerosol was demonstrated on mice, guinea pigs and rabbits (A.I. Maslov, 1960a, b). A study of the significance of this method of immunization with inactivated vaccines in protecting the organism from aerosol infection on models of pasteurellosis and mouse typhus established the following: 1)subcutaneous inoculation elicits immunological reorganization but does not save the animal's life following aerosol infection; 2) triple intravenous inoculation offers protection against ten lethal doses of aerosol infection; 3) subcutaneous and aerosol immunization caused formation of distinctly marked active immunity providing insusceptibility of animals to aerosol infection with ten lethal doses (V.M. Nikitin, 1957a, b).

L.D. Stepankovskaya (1957) arrived at a similar conclusion; she determined the most effective variant out of seven tested methods of administering heated typhoid vaccine: one subcutaneous or two inhalation immunizations at a seven-day interval.

Diphtheria, pertussis. The replacement or at least reduction of subcutaneous inoculations by aerosol immunization against diphtheria would be of great practical importance. In this connection we must mention the work of S.N. Muromtsev et al (1960) in which following primary subcutaneous inoculation with adsorbed diphtheria toxoid inhalation reimmunization was instituted. The positive results of inhalation reimmunization with diphtheria toxoid in animal experiments allowed the authors to shift to a test of this method on humans in 1961. For this purpose 113 children were reimmunized with diphtheria toxoid. An ultrasonic generator was used for

spraying. The particle size attained one micron. It was demonstrated that the inhalation method is more effective than intranasal, but is somewhat inferior to subcutaneous inoculation. No pathological symptoms were demonstrable in the children.

The efficacy of inhalation reimmunization of children against diphtheria was also confirmed by G.F. Mayorova et al (1964) who showed that reimmunization with diphtheria toxoid in a dosage of 20-37 active units consistently led to marked immunological reorganization of the organism and that revaccination of the children against diphtheria by the inhalation method is quite feasible, however due consideration should be given to allergic pathology in the past history, since repeated inhalation of diphtheria toxoid could elicit sensitization to the antigen. The possibility, in principle, of diphtheria immunization by the inhalation method was also proven experimentally in earlier works (Wolters; O.Yu. Lokotkina, 1954, 1955; Ye.A. Smirnova, M.V. Krasil'nikova, and others).

There are very few tests of aerosol immunization using pertussis vaccine. In the article by A.I. Spitsa (1960) it is stated that threefold aerosol immunization is equivalent to subcutaneous inoculation according to serological indices, although in the former case in the author's estimation the vaccine dosage was at least two-three times smaller. However actual inhalation dosage was apparently many times smaller since the atomizer yields large particle aerosols.

Tularemia, brucellosis, tuberculosis. Allergic reactions are typical for this group of infections. Therefore it was natural that an effort was made to study sensitization in response to administration of vaccines into the respiratory tract and its significance in immunity. According to skin test data the sensitizing activity of vaccines with the aerosol method of administration was weaker than with intradermal or intraperitoneal administration, but the animals' resistance to aerosol infection with virulent strains was considerably greater.

More comprehensive studies were conducted by Eigelsbach et al (1960, 1961), Saslaw and Eigelsbach, Saslaw and Carehart (1961a, b) on aerosol immunization with live and inactivated tularemia vaccine. The experiments were conducted on guinea pigs, monkeys and volunteers. The aerosol particles were about 0.7 micron in diameter. The authors concluded that aerosol immunization creates higher intensity of immunity to respiratory infection than subcutaneous inoculation, that live vaccine is more effective than killed, and that the organism's reaction to aerosol immunization is the same as to subcutaneous but is superior with respect to antibody production.

Successful studies were conducted on the use of mixed vaccines in aerosol immunization, in particular those against tularemia and VEE (W.D. Sawyer et al; T.E. Woodward and Tigertt), tularemia and tuberculosis (G. Middlebrook).

Among the studies of aerosol immunization against brucellosis we must mention that of Sh.Kh. Kharisov et al (1966) which analyzes the results obtained from aerosol and subcutaneous immunization of animals (calves). The authors indicate that the inhalation dosage of vaccine must constitute at least 32-35 billion bacteria in order to produce lasting immunity in cattle by the aerosol method. The studies on active aerosol immunization against tuberculosis are only experimental (Rauch; Schaefer et al; Cohn et al; Middlebrook; Izumi et al; Larson and Wiche; Radovic). The main purpose was to investigate the immunological response and resistance of animals in comparing the different methods of vaccine administration.

Immunomorphological Data on Aerosol Immunization

In the development of problems dealing with aerosol immunization of greatest interest are immunomorphological studies of the lungs and regional lymph nodes, but first of all we need to know the immunomorphological background of the lungs in parenteral (traditional) methods ofimmunization. Ya.L. Rapoport (1957, 1965) indicates that the proliferation of reticulo-histiocytic elements of alveolar lung septi and desquamation of alveolocytes observed with the parenteral method of inoculation create a similarity with catarrhal desquamative pneumonia which is not clinically and anatomically confirmed (organopathologically).

It is important to take into consideration this property of the lungs in aerosol immunization. Let us cite the data from the study of Yu.S. Pisarevskiy and V.A. Lebedinskiy (1965) who investigated in monkeys the morphological changes arising as the result of immunization with plague vaccine. The main changes in the lungs and regional lymph nodes consisted of specific transformation of lymphoid cells. By the 14th-18th day the lungs already failed to present any deviations from normal, i.e. all of the changes were completely reversible. The authors noted that aerosol immunization with live plague vaccine (in the doses tested) was not associated with development of destructive changes in the internal organs, which was indicative of the safety of this method.

Analysis (I.I. Terskikh et al, 1966) of immunomorphological changes that occurred on the 4th-7th day in the organs of monkeys immunized with aerosols of killed ornithosis vaccine revealed an increase in centers of follicle reproduction, high mitotic activity of reticular cells, proliferation of immature and mature plasmatic cells, macrophage reaction. Immunogenesis receded gradually: after 2-1/2 months it was still marked, but within 5-6 months it was practically negligible, with the exception of bone marrow where signs of continuing immunogenesis were detected at this time. The observed diffuse interstitial reaction of the lungs was a protective one.

The distinctions and degree of changes in the lungs in response to aerosol immunization were unquestionably influenced by the magnitude of the inhaled dose, dispersion of the sol and the preparation itself (live

or killed vaccine, its solubility, safety).

As an illustration of this position let us cite the works of White et al and Van deLoo. In both studies experiments were performed on monkeys. In 80% of the cases the dispersion of aerosols of liquid vaccine equalled two microns. The main goal was to study the immunomorphological changes following aerosol immunization. In the first study live tularemia vaccine was tested, in the second -- killed typhoid. Unfortunately the estimated inhalation doses are not comparable: 270,000 live tularemia cells, and 2 milligrams of nitrogen according to typhoid vaccine protein. Another substantial difference between the two studies was that with the use of live vaccine the vaccine strain multiplies in the lungs and scatters in the organism. Thus, on the 3rd postvaccination day there were 3 million live tularemia cells in the lungs, thereafter their number recedes rapidly, and by the 28th day they were not found in the organs with the exception of the tracheobronchial lymph nodes which "cleared up" only on the 90th day. These differences affect first of all the distinctions of pathogenesis of immunity and titer of protective antibody production, however they do not have an appreciable influence on the general picture of immunomorphological changes in the organism, except for the intensity of these processes.

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