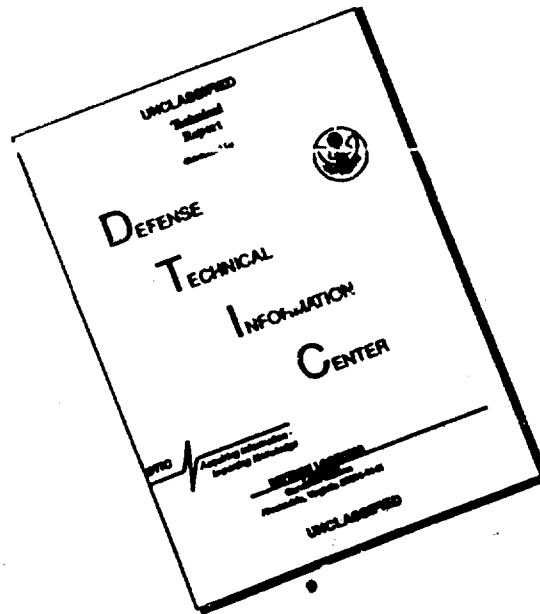TRANSLATION NO.

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casein hydrolysate agar with blood must be considered most suitable. It is also possible to use charcoal hydrolysate agar for diagnostic purposes provided 2 per cent horse blood is added to it.

Translated by A. CROZY

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ON THE MECHANISM OF SPREAD OF AIRBORNE INFECTION*

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AIRBORNE infections include a large group of diseases in which the causative organism enters as a rule through the mucous membrane of the respiratory tract. In a small number of cases infection may take place through the conjunctiva or through lesions on the skin surface, including operative wounds. Airborne infections are usually characterized by their great speed of spread and the large numbers of victims. Measures against these infections meet with great difficulties.

It had been established already towards the end of the last century that airborne infection may spread by droplets (Flügge, 1897; Lashchenkov, 1899) or with dust (Cornet, 1898). Without entering into a discussion regarding the history of this problem it should be noted that the dust theory has not found such general acceptance as the theory of droplet infection. Numerous experimental studies have been devoted to this latter problem. The intimate mechanism of spread of airborne infection, however, is still insufficiently known. This paper represents an attempt to discuss this problem from the contemporary point of view, referring to the creation and the spread of bacterial aerosols and to the ways by which these aerosols enter the human respiratory tract.

Airborne infection can take place only if pathogenic organisms which preserve their virulence are present in the air in sufficient numbers, and if this air is breathed in by persons susceptible to the infection in question. The spread of airborne infections therefore mainly depends on the following factors:

(1) Susceptibility of individuals or of a whole group to one or the other airborne infection.

(2) The presence of patients suffering from the disease in question or of carriers.

(3) The capacity of the pathogenic organism in question to preserve its virulence under the unfavourable conditions of the aerial medium.

(4) The persistence of the pathogenic organisms in the air and their concentration.

(5) Invasion of various parts of the respiratory tract by particles and droplets from the bacterial aerosol.

It appears that the first 3 factors depend on the biological properties of the macro and micro-organisms and on their mutual relationship, whereas the last 2 factors depend exclusively on the colloidal properties of the bacterial aerosol.

The main source of pathogenic organisms which enter the air are patients suffering from the disease in question and carriers harbouring the pathogenic organism in their respiratory tract.

During the physiological acts of sneezing and coughing or during the act of talking, people emit a considerable number of droplets of secretion (saliva, mucus, mucin) into the environment. As the respiratory tract contains an abundant microflora a vast number of organisms is dispersed into the air together with the droplets; these organisms inhabit the respiratory tract and particularly the oral cavity. The patient or carrier who harbours virulent bacteria or viruses in his respiratory tract emits them when sneezing, coughing or talking, and also when gargling or spitting.

It is a question of great importance which part of the respiratory tract represents the main source of organisms and in what numbers these organisms can be dispersed into the air, in other words from which sites the organisms are most likely to be excreted into the external environment.

The greatest number of droplets and consequently of bacteria is excreted during the act of sneezing. According to Duguid (1946) 4500–150,000 viable bacteria are emitted during a single act of sneezing. It is mainly saliva from the anterior part of the oral cavity, from the internal surface of the mucous membrane of the cheeks and lips, that is dispersed during the act of sneezing, and only an insignificant number of droplets are formed in the nasal cavity. Sneezing fills the air with a great number of α-haemolytic streptococci, whereas β-haemolytic streptococci are excreted very rarely even from carriers, as this organism mainly inhabits the tonsils and occurs only in insignificant numbers in the saliva.

Later Turzhetskii and Olen'eva (1957) showed that rinsing of the oral cavity with normal saline reveals the presence of haemolytic staphylococci by the hundreds and thousands, of β-haemolytic streptococci by ten-thousands, but of α-haemolytic streptococci by millions and tens of millions. From a group of 52 persons carrying on a loud conversation 347 colonies of α-haemolytic streptococci and only 1 colony of β-haemolytic streptococci could be isolated. On examination of the air of a certain room by the same authors 524 colonies of α-haemolytic streptococci and only 10 colonies of β-haemolytic streptococci could be isolated.

During the act of coughing a much smaller number of bacterial droplets is emitted than during sneezing: according to Duguid in a single cough only 910 droplets containing bacteria are emitted on the average. In the act of coughing the deeper parts of the respiratory tract are the source of these droplets. The bulk of the droplets consists of the dispersed secretion of the pharynx and secretion of the nasal cavity which has descended into the pharynx. This is augmented by some droplets from the larynx (secretion of mucous membranes brought forward by the action of ciliary epithelium from the deeper parts of the respiratory tract) and from the oral cavity.

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The number of droplets emitted during the act of talking is smaller still: only 50 droplets containing bacteria are on the average emitted by a person counting loudly from 1 to 100 (Duguid). These droplets mainly originate from the anterior parts of the oral cavity and depend on the pronunciation of certain consonants. The excretion of droplets containing bacteria was proved experimentally by Lashchenkov already in 1899.

It thus appears that only those organisms are dispersed into the air which are present in a suspended state in the secretion of the upper respiratory tract. If bacteria are firmly attached to the surface of the tonsils or if the focus of the infectious process is situated in the depth of the tissue and has little connexion with the mucous membrane of the respiratory tract the bacteria are not dispersed into the air. If, however, the bacteria can easily be washed off by saliva or any other secretion they can be excreted into the external environment within the droplets.

It is of interest that under otherwise identical conditions individual variations in the degree of salivation have a marked influence upon the number of droplets containing bacteria which are excreted from the oral cavity. Thus Rubbo and Benjamin (1955) have shown that administration of atropine leads to a fall in the number of bacteria dispersed into the air during the reading of one and the same text, a fact which can be explained by the decrease in salivation. The character of the bacterial aerosol depends to a considerable degree on the viscosity of the secretion produced by the mucous membranes of the respiratory tract. A liquid secretion, for instance, can easily be dispersed into small droplets which remain for a long time in the state of suspension, whereas a secretion of high viscosity is dispersed into a smaller number of larger droplets which settle down to the floor much earlier.

The further fate of the droplets takes the following course: around the person excreting the bacteria the aerosol reaches the highest concentration; it consists of bacterium-containing droplets of various sizes between 1 and 2000 μ; the bulk of droplets range from 2 to 100 μ. The mechanism leading to the formation of the bacterial aerosol and the transformation and mutual relation between its various phases is illustrated to a certain degree by the scheme set forth in this paper (see Fig. 1).

As a result of the kinetic energy given to the droplets by the act of coughing or sneezing, larger droplets between 100 and 2000 μ in size can be expelled over
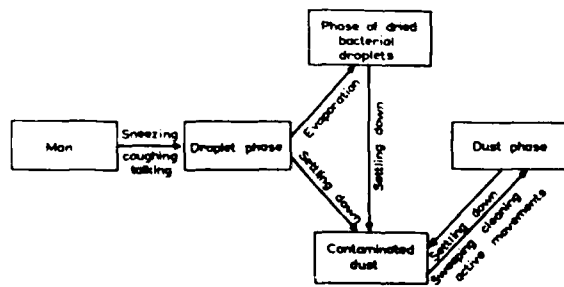

Fig. 1.

a distance of 2-3 m and more. Owing to the loss of kinetic energy the velocity of the droplets falls and they settle along a definite trajectory. The kinetic energy of small and medium-sized droplets is much lower and they are at the moment of sneezing or coughing expelled over a distance of up to 1 m from the mouth of the person in question.

Large droplets ($100-2000\,\mu$) settle very quickly (within a few seconds) on to the floor and surrounding objects, infecting the dust. These droplets gradually dry out, the bacteria become attached to dust particles and return into the air, but this time in the shape of bacterial dust. Droplets of medium size ($20-100\,\mu$) settle down much slower (after minutes and tens of minutes), and this process is further delayed by the evaporation of the external aqueous membrane and the consequent decrease in the size of the droplets. The small droplets of the bacterial aerosol ($1-10\,\mu$) can remain in suspension for a long time (many hours and even several days). Very slight aerial currents (5-10 cm/sec) are always present in any room, and around the doors, windows and heating devices these currents frequently reach velocities up to 50-100 cm/sec. It is these currents which keep the bacterial aerosol in a state of suspension, moving it around and spreading it over the whole room.

The external aqueous membrane of the bacterium-containing droplets evaporates at a varying rate, depending on a number of factors. As a result of this process the droplet phase of the bacterial aerosol turns into the phase of dried bacterial droplets. In spite of their small size the smaller droplets and the dried bacterial droplets, under the influence of gravity and of downward aerial currents, gradually settle down to the floor and to surrounding objects, where they fuse with dust particles In a certain number of cases they coalesce in the air with larger dust particles which draw them downwards, a fact which considerably increases the rate of their descent. Such bacterial dust then represents a reservoir of micro-organisms which under the influence of air currents in the room (movements of persons, shifting of furniture, etc.) again become suspended in the air (resuspension of bacteria). Enormous numbers of bacteria are suspended in the air during the cleaning (sweeping) of the premises and when beds are made (Kichenko, 1951; Kudriavtsev 1955; March and Rodway, 1954; and others). In consequence bacteria may circulate in the room (floor-air-floor) for as long as the bacteria preserve their viability and as long as the dust is present on the premises. Lowbury (1950) found that after dispersion of fluorescein in the air of a room this substance can be found for 20-23 days in a room which is cleaned daily.

Gradually the organisms die out in the dust and their virulence decreases. Many bacteria, however, retain their virulence for a long time in dust. According to Perlina (1955), for instance, staphylococci present in dust remain viable in diffuse light for 194-216 days and preserve their virulence for 129-150 days, as they are protected by a membrane consisting of serum or saliva. Streptococci preserve their virulence in artificially infected blankets for 4 weeks (van den Ende et al., 1940) and Mycobacterium tuberculosis remains viable in dust for many weeks (Effenberger, 1954). It thus appears that the time during which the dust remains on the premises can be estimated as about 20 days, i.e. a period during which many bacteria attached to the dust particles retain their viability and virulence and may freely circulate in a room which is cleaned daily.

At the time of formation of the droplet phase during the act of coughing or sneezing a rather concentrated aerosol forms in the close surroundings of the person in question. This aerosol is characterized by the great biological activity of the organisms it contains. This activity particularly includes the virulence of the

organisms, which is highest of all in the droplet phase of the aerosol. A person standing nearby can hardly protect himself against these bacteria unless the air which enters his respiratory tract is filtered through a mask. In this case the larger droplets of the aerosol reach the mucous membrane of the nose and the nasopharynx and the small droplets enter the deeper parts of the respiratory tract. From this aspect the concentration of the organisms as well as the fact that their viability and virulence have hardly been exposed to the unfavourable conditions prevailing in the aerial environment play an important part.

Bacteria in the phase of dried bacterial droplets are less dangerous, as their concentration is much lower than it was at the moment they were excreted. This decrease in the concentration is caused by the fact that a proportion of the droplets has already settled, the aerosol has been diluted by ventilation, and some of the bacteria have died under the influence of the unfavourable conditions prevailing in the aerial environment. In this phase the bacterial droplets are of smaller size and are consequently capable of entering the deeper parts of the respiratory tract. They can remain suspended for a considerable period and can be moved around by aerial currents into neighbouring rooms, corridors and staircases (Mats, 1950; Shafir, 1951).

The importance of the dust phase of the bacterial aerosols in the spread of airborne infection has not been sufficiently studied.

As has been said above, many pathogenic bacteria retain their viability and virulence in dust and can easily be resuspended into the air. Hence there is an actual danger of repeated infection during the cleaning of the room and other activities (making of beds, shaking of clothing, etc.).

It can be presumed that the importance of bacterial dust in the infection of human subjects has been underestimated.

The differences existing in regard to the behaviour, the spread and the mechanism of infection in various phases of the bacterial aerosols show the importance of a discriminating approach to the study of the droplet and the dust phases respectively of the bacterial aerosol (Rechmenskii, 1948). Particular attention should be paid to the small droplet phase of the bacterial aerosol. The difference between the droplet phase and the dust phase of the bacterial aerosol is furthermore of importance in regard to the disinfection of the air in closed premises Rechmenskii, for instance, established that the dust phase of the bacterial aerosol is much more resistant to disinfectant agents than the droplet phase.

During the process of breathing the human respiratory tract is abundantly contaminated by the aerial microflora. A considerable part of the bacteria is retained in the upper respiratory tract and only particles and droplets of smaller size penetrate into the deeper parts, including the pulmonary alveoli. The retention and penetration of virulent bacteria and filterable viruses into the respiratory tract leads to the development of the corresponding disease in susceptible persons.

The nasal mucosa represents the first obstacle or filter for the bacterial aerosol. It mainly retains large dust particles and droplets. Particles of 10–30 μ in diameter and above are completely retained in the trachea, the main bronchi, bronchi of the first, second and third order, and the terminal bronchioles. Only particles of a size not exceeding 1–5 μ penetrate into the deepest parts of the respiratory tract, into the alveolar spaces.

It should be emphasized that the respiratory tract down to the respiratory bronchioles is covered by ciliated epithelium which transports the secretion of the

mucous membranes, together with the bacterial particles and droplets it contains, towards the oral cavity. This secretion is then excreted in the form of sputum, particularly by the act of spitting, and some of it is swallowed, which may sometimes lead to infections of the gastro-intestinal tract (for this reason one of the methods of detecting *M. tuberculosis* in cases of pulmonary tuberculosis consists in an examination of stomach washings).

Infection can take place whether bacteria enter the upper respiratory tract (mainly bacterial dust) or the deeper parts (mainly small droplets or dried bacterial droplets). The resistance of the deeper parts of the respiratory tract against infection, however, is much lower than the resistance of the upper respiratory tract. Sonkin studied the influence of the size of droplets present in an aerosol of type c streptococci upon morbidity in mice. This author established that various parts of the respiratory tract are susceptible to streptococcal infection. To achieve an identical incidence, however, administration of 200 streptococci by means of inhalation was required if the size of the droplets was 1·2 μ, but more than 20,000 bacteria were required if the size of the droplets reached 12 μ. Similar results were obtained by Druett and co-workers (1956), who infected guinea-pigs with aerosols of *Brucella suis*: they found that 600 times more organisms were needed to infect the animals with an aerosol containing droplets of 12 μ size than with an aerosol consisting of 2·5 μ droplets.

Cluff, quoted by Fothergill (1957), observed a different pathogenesis of anthrax and plague in animals depending on the size of the droplets constituting the aerosol. *Bacillus anthracis*, for instance, quickly disappears from the alveoli as the organisms are transported by macrophages into the lymph nodes where they quickly multiply and enter the blood stream. Generalization of the process then leads to a secondary infection of the lungs by the bacteria. A similar process takes place if the animals are infected with an aerosol of *Pasteurella pestis* consisting of large droplets. Infection with an aerosol consisting of small droplets, on the other hand, leads to primary infection of the lung tissue without the intermediate stage of accumulation in the lymph nodes.

N. F. Gamaleia regarded the infection of the tonsils to be of particular importance, as these organs are rich in lymphatic tissue and possess numerous folds. They are situated in the path of the air current. He wrote: "One of the favourite sites of infection in the pharynx is the tonsils". Gamaleia explains this fact by "auto-infection", i.e. introduction of infectious organisms into the tissues by way of returning leucocytes.

Not only the respiratory tract but also the mucous membranes of the eye are exposed to airborne infection. Rudnev (1944) quotes an interesting example of laboratory infection: he had been working in the laboratory with *P. tularensis* for many years and had always been protected by a respiratory mask and by eye-glasses. It was sufficient to take off the eye-glasses for a few moments to wipe them as they had become covered with moisture to produce an infection. According to the experimental data of Papp (1954), the conjunctiva is much more susceptible to infection with measles, rubella and epidemic parotitis viruses than the mucous membrane of the nasal cavity or the tonsils. From these experiments the author draws the conclusion that, as far as the above infections are concerned, the conjunctiva represents a portal of entry of no lesser importance than the mucous membrane of the oral and nasal cavity. Kis (quoted by Papp) established that the capillaries of the anterior segment of the eye are capable of adsorbing liquid, and consequently infection may easily take place. The contact of the conjunctiva with infected air,

however, is relatively insignificant compared with the 12–15 m³ of air which pass in the course of a single day and are filtered in the human respiratory tract. For this reason airborne infection occurs n the great majority of cases through the respiratory tract.

As airborne infections seem to be extremely rare among wild animals Zhdanov (1953) connects the development of airborne infection with the development of human society. The development of towns, where persons are densely concentrated, and lively commercial links enhanced the development of airborne infection as early as in the slave-owning societies. Connecting the origin of airborne infection with the appearance of man and the development of human society, Zhdanov draws the unjustified conclusion that airborne infection became possible only due to the development of human speech. It is well known that in the act of talking a relatively small number of bacterial droplets is excreted compared with the acts of sneezing and coughing, which are reflex acts and in the majority of cases accompany pathological processes in the respiratory tract. Hence the main part in the spread of airborne infection is played by the excretion of pathogenic organisms during the acts of coughing and sneezing and not during the act of talking.

In view of the data quoted above certain acute respiratory infections can be divided into 2 different types depending on the speed with which they spread in a given group. Such infections as influenza or measles virus spread within a short period over the whole or almost the whole susceptible group, whereas whooping cough and epidemic parotitis spread rather slowly and do not cause illness in all susceptible persons. In the case of influenza and measles the illness is accompanied by an abundant excretion of mucous liquid secretion which contains the causative organism The increased reflex excitability of the respiratory tract leads to sneezing and coughing, and in consequence the aqueous secretion is easily dispersed in the form of a great number of small droplets. In pertussis, on the other hand, the secretion possesses a high viscosity and is dispersed with great difficulty into a relatively small number of large droplets which quickly settle down to the floor (Kasparova, 1957). Parotitis is rarely accompanied by inflammation in the upper respiratory tract and consequently with sneezing or coughing. Besides, parotitis leads to a marked fall in the secretion of the affected salivary glands, a fact which delays the penetration of the virus into the oral cavity. Hence, in whooping cough or parotitis a much smaller number of droplets containing bacteria or viruses is dispersed into the air than in influenza or measles. This fact prevents to a considerable degree the spread of infection among susceptible pe sns. The difference in the course taken by outbreaks of measles and parotitis respectively in children's communities was well described in the paper of Kasparova.

Along with the difference in the way the bacterial droplets enter the respiratory tract it should also be taken into account that it takes various organisms a different time to die under the conditions of the aerial environment. This problem, however, is at present insufficiently studied.

Finally, it should be emphasized that each of the various types of airborne infection has its peculiarities in regard to its spread, a fact which requires further investigation. For this reason a profound study of the biological properties of the causative organisms in various phases of the bacterial aerosol and the mechanism leading to spread and infection might well help to find more effective measures against airborne infections.

Translated by F. S. Freisinger

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EXPERIMENTAL STUDY OF COMBINED VACCINATION AGAINST PLAGUE AND TULARAEMIA*†

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IN the literature there are reports on the successful use of combined vaccines con-
sisting of killed bacteria and toxins against such infections as tetanus, diphtheria,
scarlet fever, typhus, cholera, dysentery, etc. A still insufficient amount of work
has been done on the possibility of using combined live vaccines and live vaccines
in association with killed ones.