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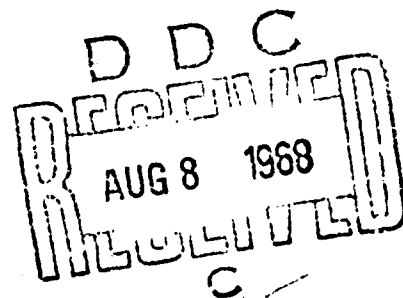
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Special Conditions for the Penetration of Infective Pathogens Through
the Intact Pulmonary Surface

by H. Buchner

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The preceding experiments have demonstrated, as a general result, the penetration of intact surfaces of the lung by certain infective pathogens. In the ensuing, a few specific points will be subjected to detailed discussion. The principal questions are: How is perforation of the lung accomplished?; Which conditions favor it and which conditions prevent it?; Which types of infective pathogens suggest the possibility of penetration, and which exclude it?

a). The manner and method of penetration through the pulmonary surface.

Permit me to start with a compact perspective on inhalation and feeding tests and the principal results obtained with anthrax, fowl cholera and septicemia.

	Inhalation				Feeding		
	Number Inhaled	Succumbed to Anthrax etc.	Succumbed to Pneumonia	Survived	Number Fed	Succumbed to Anthrax etc.	Survived
Dry Atomization Anthrax Spores	63	49	5	9	33	4	29
Wet Atomization Anthrax Spores) Anthrax Rods)	27	23	-	4	10	3	7
Fowl Cholera) Rabbit Septicemia)	50	24	-	26	36	-	36
Total	140	96	5	39	79	7	72
	= 68,6%				= 8,9%		

Although it is comprehensive, this statistical total result has only limited value. It shows nevertheless that in our tests inhalation was considerably more dangerous than feeding, the ratio being 68.6 to 8.9. This ratio should not be considered strictly valid in the sense that inhalation is actually about 8 times more dangerous than the feeding of the same pathogen. One cannot draw this conclusion, since in most feeding tests the amount ingested was much too large and far greater than would have been necessary for comparison. I refer in this connection to test No. 28 with feeding of maximal amounts of spores, in which 3 guinea pigs expired. This single test raises the ratio of animals by feeding from 5.1 to 8.9%.

According to this statistic, feeding is presented in too unfavorable a light; conversely, inhalation is shown to be too favorable, in which connection I recall inhalation tests with minimal amounts of pathogens in which the lower limits of the efficiency of this mode of infection was determined. If a large number of inhalation tests with somewhat greater quantity of anthrax spores were to be initiated, one could certainly obtain 100% mortality. However, what would be the purpose of such an undertaking?

In earlier chapters I have pointed out several times that such a statistic, even if resulting in 100% mortality, would not suffice for satisfactory proof of the penetration of the lung. We therefore attempted to furnish direct proofs and we were successful in this. I refer the reader to examinations of the lungs of inhalation animals by means of plate cultures, as described in Chapter 2, which were carried out in numerous cases and which demonstrated that multiplication of numerous inhaled pathogens had taken place in the lungs.

The most direct proof for the penetration by the pulmonary route was furnished by the microscopic examination of the lungs of inhalation animals, which had been sacrificed 20 to 24 hours after inhalation, that is, at the time of commencing infection. In the fact that this demonstration was carried out in two entirely independent series, i.e., by dry atomization and inhalation by the wet route in an identical manner, I recognize unequivocal proof of the perforation of the pulmonary surface by infectious pathogens.

The preparations obtained during the first test series (plate 1, figure 2) differed decidedly from those obtained through wet atomization (plate 1, figure 4) by the circumstances that in the former we could not find enclosure of anthrax bacilli in blood capillaries, whereas it is precisely this finding which characterized the preparations of the second test series. Thus we are apparently dealing with two different, successive stages of the process, which here appears before our eyes in its details. First the anthrax spores germinate, then there is a stage in which small groups of bacilli are found on the alveolar wall and in certain layers, also under the alveolar epithelium. In the second stage there are isolated

focal accumulations of larger quantities of anthrax bacilli in the pulmonary tissue, and these bacilli are for the most part already enclosed in the passages of the blood capillaries.

The question is: How and by which route has this transfer to the blood passages taken place? Only three possibilities suggest themselves, one of which must be considered impossible. We shall nevertheless discuss it in a few words.

One could think first that the bacilli found in the pulmonary capillaries do not originate with spores taken up into the alveoli during inhalation, but come from others which were accidentally swallowed during the inhalation test period. This is contradicted principally by the short interval (23½ hours) between inhalation and sacrifice of the animal. The test with maximal spore feeding of guinea pigs had shown that all animals succumbed only after the fourth day. It is impossible, therefore, that respectable foci are produced in the lungs already after 23 hours, if they emanated from the intestines. If maximal amounts of spores require that much time to produce an infective process in the body after travelling through the stomach, the minimal quantities which are possibly swallowed by accident cannot produce the same in half the time. At any rate, the possibility of infection from the intestine with such minimal quantities must be negated absolutely. Moreover, plate cultures of the spleen showed complete absence of anthrax bacilli, suggesting the sterility of the large circulation and the total organism. Thus the assumption of invasion of the lung from another site, especially from the intestinal canal, must be rejected categorically.

This only leaves the possibility that bacilli found within the capillaries originate directly from inhalation spores. There are two possibilities: either this involves transportation by the lymphatic route to the bronchial nodes, from which the germs reach the blood and the small circulation, or there is direct transfer into the blood stream.

These two possibilities are not mutually exclusive. Both routes may be taken simultaneously. The lymphatic route is supported by Muskatblueth's results, who was able to demonstrate copious bacilli in the bronchial glands 17 hours after injection of anthrax bacilli into the trachea. Additional support is given by Arnold's results with dust inhalation, discussed in Chapter I, in which the movement of inhalation dust particles to the bronchial gland could be demonstrated within a few hours.

A rapid passage of inhalation anthrax spores or rods into the bronchial glands therefore is undoubtedly possible. One may ask, however, how long it takes until passage into the blood stream is accomplished. Muskatblueth claims to have found an answer to this question. He reports that animals who succumbed to tracheal anthrax infection after 48 hours no longer revealed anthrax bacilli in the bronchial glands. Consequently

these must have passed on and entered the blood stream. It is clear, however, that such a negative finding does not yet permit a certain conclusion. It would be possible that anthrax bacilli still demonstrable in the bronchial glands 17 hours after injection are no longer found 30 hours later because they had died in the bronchial glands. Muskatblueth postulates onward migration only because he later found bacilli in the blood stream. However, this result does not prove the assumed correlation, since another possibility exists for the passage into the blood stream.

Concerning the conditions in connection with dust inhalation, these definitely speak against rapid passage through the bronchial glands. Here the interval between intake of dust particles into the bronchial glands and release from the latter is infinitely great, i.e., the bronchial glands function as completely impassable filters. In my opinion this does not apply to infective pathogens, as already discussed in Chapter 1. One should not believe, however, that passage is so easy that it is accomplished within the span of a few hours; certain morbid changes in the lymphatic glands will be required in order to make passage possible. At any rate, this agrees with our clinical experience concerning the involvement of lymphatic glands during infected processes in man.

On the basis of these considerations I must postulate that it is impossible for anthrax bacilli found in the blood capillaries in our preparations to have reached the small circulation via the lymphatics and the bronchial glands. The short time available for this process seems to contradict this decidedly. The size of foci found in the lung proves that multiplication had occurred here for several hours.

This only leaves the possibility of direct passage of bacilli grown out of inhaled spores into the blood stream, and this opinion is indeed the most likely and, in my opinion, the only valid one. It would be begging the question if the process is explained in any other way. All of our investigations show that the spores reach the alveoli during inhalation; the fact that germination occurs here is proved by microscopic preparations from tests with dry spore inhalation. Since we find large foci in the pulmonary tissue and in the blood stream already 23½ hours after inhalation, we must conclude that passage had taken place in situ. It may be postulated at least that a more direct proof for passage can never be established. As already noted in the preceding chapter, the instant of passage itself will never be perceived with any degree of reliability.

After we have obtained positive anatomical support for direct passage into the blood stream, we must ask whether the assumption of such a passage represents something so unusual that one can only decide on it as a last resort, that is to say, in an emergency. This is not the case. On the contrary, when the properties of the blood capillary walls on one hand and the properties of anthrax bacteria on the other are considered, the probability of such a passage is suggested from the start. This does not involve

drilling through the wall as had been assumed in the past, but represents growth through gaps which form in the vascular wall under the influence of morbid irritants. The same gaps which permit passage of leucocytes and red blood cells in connection with every inflammatory irritation, apparently due to retraction of the endothelial plasma, must also occur when a pathogen exerts its chemical activity in the close proximity of the vascular wall. The production of toxic substances must here effect the same in a minimal location which otherwise is elicited as a rule by the inflammatory irritation over larger areas. Even if only the smallest of gaps exists, bacteria with their extraordinary minuteness may grow through.

The fact that this penetration, in the final analysis, is based on an active function of the bacteria, i.e., on growth and reproduction, is the reason why inhaled coal particles, etc., can never enter the blood stream. There just is no liquid flow and no chemical transportation in this direction. By the same token nonpathogenic bacteria, even if they have reached the alveoli, can never pass through the capillary wall because they are unable to propagate in the tissues and fluids of the body. Moreover, they normally are ingested by phagocytes before a slow reproduction can take place and are removed in this manner.

In order to eliminate any misunderstanding, I want to stress once again that this assumption of a direct passage into the blood stream does not exclude the other route via the lymphatics and the bronchial glands. Both routes may be used simultaneously. The transport via the lymphatics into the bronchial glands, which is a purely mechanical process, must also be possible in the case of pathogenic bacteria, as well as in the case of lifeless coal splinters, cinnabar granules, etc.; the tests by Muskatbleuth have proved that transportation of anthrax bacteria up to that point actually takes place. Similarly, nonpathogenic bacteria which occur as dust in the ordinary atmosphere may be introduced as far as the bronchial glands. But here a great difference will become apparent; lifeless dust and nonpathogenic bacteria are held back completely, in which connection the latter will sooner or later be killed and absorbed, this fate is not always incurred by pathogenic bacteria and not always to the same extent. It cannot be denied, therefore, that such a route of perforation exists for pathogenic bacteria; we had to determine which route must be assumed in our case, in our preparations, as the actual one, and this can only be that of direct passage into the blood stream, based on the preceding considerations.

The passage of bacteria through the intact pulmonary surface is always and under all circumstances an active process. This sentence may be considered one of the most important results of our experiments. To this extent, Fluegge is right, when he designates the "intact" pulmonary surface as impassable. For in the same instance when active penetration takes place, this "intactness" is eliminated. Purely mechanical transportation and passage does not occur; all portals are closed if the pulmonary surface is intact, both for nonpathogenic bacteria and for lifeless dust. There would be no advantage in defining "intactness" in this sense.

On the other hand, the concept of "pathogenic" bacteria necessary requires closer definition with regard to these conditions, since faulty understanding of it has already created confusion and undoubtedly will cause more of it.

Fluegge and his pupil Wyssokowitsch, as well as Laehr in recent times, who under the supervision of Ribbert has conducted elegant and instructive tests, have introduced "pathogenic" bacteria to the pulmonary surface and still could not observe perforation. The "pathogenic" bacteria with which they experimented were *Staphylococcus pyogenes aureus* and *typhus bacilli*; rabbits served as test animals. It is a fact that *typhus bacilli*, if introduced in large quantities into the blood stream or the peritoneal cavity of rabbits, cause death of these animals. But where is the proof to the effect that these bacilli multiply in the rabbit's organism under such conditions? One has, on the contrary, come to the general conclusion that it is only the toxic effect to which the animals succumb. Even if slight propagation takes place in the animal, which is weakened by intoxication after injection of a large quantity of *typhus bacilli*, this would still not prove that individual *typhus bacilli* are capable of propagation without the supporting influence of intoxication. One cannot expect that individual *typhus bacilli*, which reach the lungs during inhalation, are capable of active passage into the blood stream. Tests of this type with an animal species not susceptible to *typhus bacilli*, as is the case with rabbits, only promise a negative result from the start, and it is no wonder that Fluegge obtained this result.

The conditions are very similar in the case of *Staphylococcus pyogenes aureus*, which is pathogenic for rabbits, but only after installation of somewhat larger quantities. In this case propagation within the animal may, without a doubt, take place; the result is either local formation of abscess with copious propagation of *staphylococcus* in pus, or the well-known localizations in the kidney where multiplication may also occur. However, small amounts or even isolated cells of *staphylococcus* are not able to produce propagation and infection in a healthy rabbit. The investigations of Grawitz and de Bary have produced new proof thereof ^{1/}; these researchers found that *staphylococcus* is only capable of existing and reproducing in the organism if the substrate has been prepared for its settlement by its own decomposition substances or other suitable agents (*Liq. ammon. caust.* or decomposition products of *Bacillus prodigiosus*).

^{1/} P. Grawitz and W. de Bary: Concerning the causes of subcutaneous inflammation and separation. *Virchow's Archive*, 1887, Vol. 108, Issue 1, page 67.

Thus *Staphylococcus aureus* is not infectious for rabbits in the higher sense, such as would be the case with anthrax or fowl cholera bacilli, each individual of which could, under certain conditions, lead to an infection. For other, more susceptible animal species, the situation might be different, as also in the case of man, especially sick persons, if the conditions resemble those produced in the rabbits by injection of ammonia, etc. But in the healthy, intact rabbit isolated staphylococcus cells will show no reproduction and for this reason will be unable to pass the intact pulmonary surface by an active function. Fluegge and Laehr's negative results with staphylococcus with respect to penetration of the lung are readily explained thereby.

b) Conditions which prevent passage through the lung.

Another point in the investigations of Laehr is relevant. These were carried out not by inhalation but by injection of staphylococcus suspensions in the trachea (3 cc each). While this is apparently a very mild process, it nevertheless produces irritation if the fluid reaches the fine bronchial ramifications and is aspirated to the alveoli, especially if this fluid contains decomposition products of staphylococcus even in small amounts. As early as 8 hours later, Laehr found all staphylococci in cells, partly in leucocytes, partly enclosed in epithelium. This copious extravasation of colorless blood cells and desquamation of epithelium must definitely be attributed to the irritation produced by injection of fluid. It is quite clear that the chances for active invasion of the blood stream by staphylococci are even more reduced thereby. Indeed, Laehr has offered the interesting and important proof that staphylococci ingested by phagocytes die rather rapidly, especially in the epithelium.

The greater the irritation of pulmonary tissue, the smaller is the prospect for a passage through the lung. This agrees well with the active character of the passage. Proof of this was offered already in Chapter 2, which involved the genesis of pneumonia after inhalation of dry spores. Additional support was offered by tests described in Chapter 3, involving inhalation of anthrax rods. The stimulation of pulmonary tissue was not mechanical but infectious, caused by the mass of bacilli acting at once. The result was a highly intensive pneumonia followed by an unusual delay in total infection of the body with anthrax, in spite of the colossal amount of bacilli present in the lungs. I refer to the findings listed there, according to which the spleen in these animals was very small and contained only isolated anthrax bacilli. One would expect the opposite, e.g., that here, where inhalation introduced such large masses of anthrax bacilli into the lungs, causing such a violent primary localization, passage into the blood and the total organism would occur quite early and copiously. But this assumption would have been false. On the contrary, pneumonic irritation apparently inhibited and delayed passage. These viewpoints also explain the negative results of recent tests by Hildebrandt, who conducted extensive inquiries into the behavior of infective organisms

which penetrate the air passages ^{1/}. According to a report by Baumgarten (Pathological Mycology, 2d half, page 455), these tests did not succeed in infecting rabbits by intratracheal import, even when relatively colossal amounts of anthrax bacilli or spores were involved; although guinea pigs usually contracted anthrax after identical application, the positive results were contestable since the occurrence of disseminated anthrax of the throat tissue could not be prevented due to violent and consistent regurgitation of injected fluid. Based on this description one cannot doubt that the quantities of anthrax fluid used in these tests were excessive. Although generally speaking the rule is correct that large amounts may still be effective where small quantities fail, this inference is not valid everywhere, not even in the field of the infective doctrine. If inhalation of anthrax bacilli suspended in the air produced such a vigorous stimulation that severe pneumonia resulted and that passage of bacilli into the blood was considerably impeded, it is certain that in the tests of Hildebrandt, where persistent regurgitation of anthrax fluid occurred, this irritation became so intensive that it explains satisfactorily the complete prevention of passage through the lung. The difference between these tests by Hildebrandt and those by Muskatblueth must be found in the injected quantities which amounted to only 0.2 to 0.3 cc, according to the latter researcher. This probably is the maximum which still promises positive success in passage.

Experiments with anthrax carried out heretofore also confirm the doctrine based on the investigations of Laehr and Ribbert, that the inflammation of the lung constitutes a process which is beneficial to the total organism by preventing the invasion of pathogens through the lungs into the interior. In any case, the activity of phagocytes plays a major role, although doubtless there are other changes in an inflamed tissue which are capable of preventing the propagation of invading bacteria. However, phagocytes will appear more numerous and energetically if the inflammatory stimulation is intense. For this reason the inhalation of isolated germs, which begin their multiplication on the alveolar wall without any stimulating side effects, and which may enter the blood stream, may in many cases constitute a much more dangerous mode of infection than injection of larger amounts of the same pathogen, if a violent irritation is produced. Even in the case of inhalation there will be a limit, that is, an optimal effective quantity, beyond which the resultant irritation again impedes success with regard to passage through the lung. Our tests with inhalation of anthrax rods prove this, and test number 40 with fowl cholera bacilli also shows that the inhalation of large amounts may produce inflammatory stimulation of the lungs which, without a doubt, may exert an inhibiting effect upon increased and general dissemination. This is less important upon inhalation of spores, since these cannot produce the irritating effect as long as they have not yet germinated. However, upon employment of the vegetative state of pathogenic fission fungi in inhalation tests which are to demonstrate passage through the lungs, one

^{1/} This report has not yet been published.

should observe test conditions with respect to quantity which we have used in air tests, if favorable results are to be obtained.

It is in complete agreement with these considerations that we have seen no signs of irritation, no phagocytes and no inclusion of anthrax rods in cells in our pulmonary sections from the stage of commencing anthrax infection in the case of spore inhalation. These inhalation tests gave such a prompt result precisely because no irritation occurred and because the infection entered the organism of the animal by stealth. Actually the inclusion of bacilli in rods is not a normal manifestation of anthrax in the guinea pig; the rods are always found to be exposed in anthracic animals and no phagocytosis was observed. Inclusions can form only in connection with a mode of infection involving irritation, and this is the case when anthrax rods are introduced into the pulmonary tissue together with an irritating fluid, as was the case in Muskatblueth's experiments. Here we found numerous cellular inclusions because inflammatory manifestations are produced and because a considerable portion of these rods, which were introduced from without and did not grow in the animal organism under local conditions, are unable to resist phagocytosis. The latter therefore are ingested by phagocytes and are eliminated from the infective process. The free rods, on the other hand, of which Muskatblueth found a considerable number, are able to carry on reproduction and to initiate the infective process.

- c) Which types of pathogens are suited for perforation of the intact pulmonary surface?

We finally have to take up the question of pathogens other than anthrax and fowl cholera bacilla, etc., which probably are able to pass through the intact pulmonary surface.

Most eminently suited for this purpose are the blood parasites, i.e., those pathogens which live in the blood and are capable of reproducing in it. The reason is obvious, since these alone are capable of effecting direct, active passage into the blood stream of the lung as has been demonstrated in these experiments with respect to anthrax bacilli. All other infective pathogens are completely excluded from this mode of transfer. How else could bacteria penetrate the capillary vessels of an unimpaired tissue if not by growing through gaps in the walls, i.e., by reproducing in the blood itself, even though on a minimal area?

This category of blood parasite includes principally the anthrax bacilli, the bacilli of mouse and rabbit septicemia, and of swine erysipelas. In the case of all these the possibility of direct passage into the pulmonary capillaries undoubtedly exists in all susceptible animal species. In the case of anthrax bacilli special attention should be directed to this factor in view of the natural genesis of anthrax in cattle, horses, goats and sheep; in the case of erysipelas bacilli, in view of the natural genesis of swine erysipelas. It would be worthwhile to initiate such inhalation tests with

larger animals employing anthrax spores. Their positive results can be predicted in advance, and the only difficulty would involve intestinal infection due to accidentally swallowed spores, which may be eliminated positively in the case of rodents, even in the case of these animals which are more susceptible to ingested anthrax. The method would again consist in determining that a certain small quantity of spores is effective when ingested, while the same amount would induce a lethal anthrax infection upon atomization and inhalation. It is quite probable that a much smaller quantity of spores than used in feeding would suffice to induce a respiratory infection in ruminants, as in the case of rodents. In addition, direct proof of pulmonary infection must be provided.

As long as such tests with ruminants, especially with sheep, have not been carried out, the absolutely positive proof of infection of these animals by the respiratory route is lacking. However, we can infer by analogy that this possibility exists. We may ask whether a considerable percentage of cases involving spontaneous anthrax might be explained by this fact. There are many occasions for the atomization of anthrax spores by the wet and dry method and for subsequent inhalation. One could object that the question of spontaneous anthrax has already been decided and that it had been proved to be intestinal anthrax once and for all.

The available evidence for this is not convincing, however. It is true that the investigations of Koch, Gaffky and Loeffler ^{1/} established in the case of some animals which succumbed to spontaneous anthrax (during barn feeding) that lesions in the intestinal wall were present, and it was determined in one case that anthrax infection had taken place thereat, as in the case of sheep who were infected experimentally with ingested spores. This observation material is small, however, and would permit conclusions about the behavior of spontaneous anthrax only when all other routes of infection have been excluded, especially in view of the peculiar dependence of larger epizootics on chronological and local factors.

Even the frequent occurrence of intestinal localization in spontaneous anthrax does not necessarily prove genesis from the intestine, since secondary metastatic, carbuncular affections of the intestinal mucous membrane may develop. It is known that carbuncular foci are frequently seen on the mucous membranes of the digestive canal even in persons who died of the effects of cutaneous anthrax; these foci are certainly of metastatic nature, since, in addition to the intestinal foci, we occasionally find similar foci in the mucous membrane of the stomach which, according to our present knowledge, can only have developed through the agency of the blood stream. It is possible that many cases of apparent intestinal anthrax of the larger animals develop carbuncular foci of the intestinal mucous membrane by the same metastatic route.

^{1/} Reports of Imperial Public Health Service, 1883, Vol. 2, page 147.

In the case of blood parasites, the location of entry into the body, that is into the blood stream, is not always marked by macroscopic changes. This is particularly true of infections by the respiratory route. We have seen many times that inflammatory lesions of the pulmonary tissue act, on the contrary, by preventing passage of pathogens into the blood. Bollinger had compared pneumonia induced by inhalation of massive anthrax rods with an anthrax carbuncle, and it was precisely this pneumonia which impeded and delayed infection of the total organism, while in all the numerous cases of spore inhalation of fowl cholera bacilli, where no visible changes were seen at the point of entry, passage and infection of the total organism occurred with great facility and speed. The current doctrine, according to which the site of infection by a microorganism is usually marked by gross, visible lesions, therefore has no general validity.

We now come to the blood parasites which act as infective pathogens in man. Again the anthrax bacillus comes to the foreground; in the case of so-called "rag-pickers disease" it is probably taken up through the lungs, especially in those cases where all perceptible signs of entry at another site (cutaneous or intestinal carbuncle) are absent. In addition, we must consider relapsing fever and malaria. These spirilla of relapsing typhus are exquisite blood parasites; the possibility of their direct passage into the blood stream, if they reach the alveoli by inhalation, must be assumed. Malaria also involves a blood parasite, e.g., coccidium, one first described by Marchiafava and Celli; which lives in the interior of red blood cells and which is recognized by counter-staining as an independent organism and not as a mere manifestation of degeneration, as seen in Metschnikoff's preparations ^{1/}. If this parasite inhabiting the blood is indeed the cause of malaria, the possibility of direct passage of the pathogen into the lung's blood stream can be assumed, and this would agree very well with our epidemiological experience with respect to malaria, which definitely speaks against assimilation of the infection by the digestive route and which supports the respiratory route.

The bacilli of tuberculosis and glanders, which show numerous analogies in their behavior, are not blood parasites. However, the bacilli of tuberculosis may occasionally be found in the blood of highly tuberculous persons and highly infected experimental animals. The proof of multiplication within the vascular system is lacking; apparently we are dealing with mechanical transportation of bacilli, since they cannot be found in massive quantities

^{1/} Certain negative evidence also speaks in favor of these endo-cellular parasites, e.g., the fact that specific bacteria are absolutely absent from the bodies of malaria patients and especially from their blood. The demonstrated infectiousness of the blood during transmission to healthy persons (Gerhardt, Marchiafava, and Celli) must necessarily be attributed to these coccidia which cannot be cultivated in our media as animal parasites.

within the blood vessels. It is true that tubercles are seen in the intima of larger veins. But these prove that penetration by growth into the neighboring blood stream is not easy or does not occur at all.

Consequently we cannot assume direct passage of aspirated bacilli of tuberculosis or glanders into the pulmonary blood streams. Inhalation experiments with TB bacilli have confirmed this long ago, since general infection of the animal organism does not occur immediately; instead, a localized tuberculosis infection develops in the lungs. Localizations in internal organs develop secondarily, but most probably not by direct passage of TB bacilli into the pulmonary blood stream, but rather by transport along the lymphatics and by successive tuberculosis infection of intrapulmonary lymph follicles, followed by involvement of the bronchial glands from which fully virulent bacilli may pass into the blood of susceptible animals. Baumgarten points out correctly that this passage through lymph glands is not a simple mechanical transport but that the infectivity of TB bacilli plays the decisive role. This explains why human tuberculosis rarely involves early localizations in internal organs, as would be the case if TB bacilli were constantly passing from the lung into the circulation. The path through the bronchial glands is relatively difficult and slow. Lifeless particles and apathogenic bacteria cannot pass at all. We have already discussed the active function of the microorganism which is needed for this type of penetration. In human pulmonary TB, passage by the lymphatic route occurs as the rule only if the vigor of the organism fades gradually in the course of prolonged sickness; susceptibility of the organism is then slowly increased while transportation of bacilli by the lymphatic route becomes increasingly more frequent. Under these conditions isolated bacilli succeed in overcoming the barriers, in fending off the destructive attacks of phagocytes and possibly in producing limited dysfunction of impeding lymph glands in a small area, which then permits penetration into the vasa efferentia.

This discussion also shows that primary localization in the pulmonary passages is not needed for possible penetration of aspirated TB bacilli through the lung. Since this perforation is not direct, but occurs by the lymphatic route and therefore depends exclusively on the difficulties and events of transportation through the lymph glands, the passage of aspirated TB bacilli into the internal organs without local pulmonary TB can be well imagined. Some clinical experience with primary tuberculosis of internal organs support the actual occurrence of this process.

In the case of glanders bacilli, where knowledge concerning these things is generally more sparse, I was able to carry our experimental confirmation, which apparently speaks for such a correlation.

On 3 February 1888, two guinea pigs were subjected to inhalation of atomized glanders bacilli (agar culture suspended in 40 cc of water) for $\frac{1}{2}$ hour, in the manner used in Chapter 3. After 6 days both animals showed

audibly impeded breath and brownish secretion at the nostrils. Both animals died, one after 14, the other after 39 days, accompanied by marked emaciation. The sectional findings, confirmed by culture and bacteriological tests, were very peculiar in both cases. The findings in the animal which died 39 days after inhalation will be listed briefly:

Marked meteorism. No external signs at the nostrils; during opening of the nose passages we found purulent adhesions on the mucous membrane but no nodules or scars. The axillary glands on the right are somewhat swollen but normal on the left; inguinal glands on both sides somewhat enlarged. The lungs show natural color and condition. In the right superior lobe there is a strongly injected area slightly larger than a pea, which feels coarse. Cross section reveals a wedge-shaped, light yellow infiltration consisting of different confluent foci. One tracheal and one bronchial gland are enlarged. The spleen is enlarged, especially in the transverse section, and has normal coloration. On the surface and on the cross section there are relatively numerous miliary, light yellow, prominent nodules. The liver is normal. The kidneys are hyperemic, otherwise normal. The stomach and colon are distended meteorically; the former contains some aqueous fluid. The mucous membrane of the entire intestinal canal is unimpaired and in a normal state; this is true also of the mesenteric gland.

Of these findings I consider most noteworthy that the lung contains a single circumscribed, fairly large glanders nodule in the right superior lobe, while the remaining lung was normal. Since inhalation undoubtedly conducts glanders bacilli into many parts of the lung, most of them must have remained ineffectual. Had the test been made with more virulent material than was available, the effect probably would have been more generalized, similar to inhalation of TB bacilli. By chance, favorable conditions for settlement of glanders bacilli existed only in the right superior lobe; perhaps a somewhat larger quantity had reached that point. If this favorable coincidence had not happened, perhaps the whole lung would have remained free of glanderous eruptions. This leads to the question why the demonstrated glanderous affections of the spleen had developed in this case, and whether the glanderous nodule in the right superior lobe constitutes the link between inhalation and splenic affection.

This is answered unequivocally by findings obtained from the animal which died earlier, that is, after 14 days, since here the lung is completely intact and the presence of beginning miliary eruptions of glanders in the spleen could nevertheless be demonstrated. I shall leave aside the detailed findings in this case and mention only that glanders bacilli could be confirmed in the spleen by culture and that there were no signs of infection emanating from the intestinal canal. The latter possibility should be completely discounted, considering the extremely low susceptibility of guinea pigs for glanderous infection of the intestines and the low virulence of the culture employed. This leaves only the assumption of an infection emanating from the lungs, of which I can only assume

that it developed by the lymphatic route independently of any primary localization in the pulmonary tissue. The details of this process must be investigated in the future. We were unable to find a greatly enlarged bronchial gland in our case. There can be no doubt, however, that the glands constituted the site of passage. Thus a primary localization in the lung is not *conditio sine qua non* for the passage of inhaled glanders bacilli into the blood and the internal organs, and one may assume that such is possible also in the case of TB bacilli, and that primary tuberculosis of kidneys, testis, or bones, can develop through inhalation, even if localizations in the pulmonary tissue are completely absent.

Of the remaining infective pathogens of man, the cocci of erysipelas and pus occupy an intermediate position with respect to the problem discussed here. They cannot be considered genuine blood parasites nor can their ability to reproduce in the blood be negated. In the case of the coccus of erysipelas, the concept according to which their multiplication occurs only in the lymph spaces and the lymph vessels is not correct, since Hartmann ^{1/} demonstrated these cocci in dense masses in the liver capillaries in fatal human erysipelas. Apparently this involves a secondary process made possible by the morbid course and the lower resistance of the organism. Primary proliferation of erysipelas should not occur in the blood, and the coccus should not be suitable for direct passage into the pulmonary blood stream in healthy persons. Whether the passage through the lymphatics and the bronchial glands is possible cannot be decided; such a process may be assumed in view of the special ability shown by the cocci of erysipelas for propagation in lymph vessels and lymph glands. In that event such cases of spontaneous mycotic peritonitis, as described by Hartmann, could be explained in this manner; copious cocci of erysipelas were found in purulent exudate in the abdominal cavity without accompanying cutaneous erysipelas.

The situation is somewhat clearer in the case of *Staphylococcus pyogenes aureus* and *Streptococcus pyogenes*, since both excitors of suppuration have been proved to reproduce within the blood circulation in view of their active participation in endocarditic processes. Their stability is large enough to permit experimental production of endocarditic processes in the rabbit which normally is less susceptible to this pus pathogen than man. *Staphylococcus aureus* is found to be situated freely on the surface of valves in-midst the blood stream; the streptococcus is found more in the tissue of valves. In the case of the latter, the frequent finding of capillaries of the kidney and other organs filled with cocci in pyemia offers proof of propagation in the blood.

^{1/} On the etiology of erysipelas and puerperal fever. This Archive Vol. 7, page 83.

The ability of pus cocci to exist in the living blood and, consequently, the possibility of direct active passage into the blood stream therefore cannot be denied, especially in the case of man. In the rabbit this would not be as easy for reasons already mentioned; experiments to this effect therefore have little promise of success. A gradual penetration by way of the lymphatics and the bronchial gland would be more likely. In the sick animal, on the other hand, the conditions could be different, as they ought to be in the sick person, where penetration could take place under certain conditions.

Finally, mention should be made of the typhus bacillus and the cholera vibrio. Again these are not genuine blood parasites. However, as generally expected, the former may form its settlements in the blood capillaries of internal organs of typhus patients, suggesting a certain ability to grow within living blood. It is not certain whether this points to direct passage of inhaled typhus bacilli into the pulmonary blood stream of man. As long as no animal species susceptible to typhus are available, such animal experiments offer little promise of success. Even if passage should occur, isolated bacilli will soon die in the nonsusceptible animal organism and cannot be demonstrated. Since the process of penetration is an active one, we cannot assume that this event takes place. As a matter of fact, Fluegge and Wyssokowitsch obtained negative results after instilling typhus bacilli in the trachea of animals, as could be expected. This experimental inaccessibility must be deplored in view of the epidemiological behavior of abdominal typhus which points to a correlation of typhus with certain processes in the soil, something we cannot explain through intestinal infection for the time being.

In the case of the cholera vibrio the situation is similarly unfavorable or even more so, inasmuch as we get no information about the relation of this infective pathogen to the living blood by way of cholera patients. With the exception of scant data by Babes, who demonstrated cholera vibrios in the kidneys of cholera cadavers, they have heretofore been found only in the interior of the intestinal canal as well as in and on its walls. For this reason we are limited to animal tests and to examinations of blood withdrawn from animals under aseptic precautionary measures.

I have carried out tests of the aforementioned type recently and a year ago. The method for procurement of blood is as follows: after complete disinfection of the appropriate skin area, the carotis of healthy rabbits is exposed with sterile instruments and a sterile canule is inserted in the vessel after temporary blockage of blood supply by means of a clamp. The outflowing blood passes through a sterile rubber hose, which is attached to the canule into a sterile glass vessel which is equipped with glass pieces for the purpose of defibrination. After defibrination the blood is taken from this flask and distributed by means of sterile pipettes on an arbitrary number of sterile, flat flasks which are closed with cotton. This yields a completely sterile nutrient which, when stored

in a cool place and protected against evaporation, remains unchanged for at least 14 days. It also remains intact for about 1 week in the incubator. Dissolution of blood cells begins gradually and the blood becomes more and more lake-colored.

When such sterile blood, contained in flat flasks, is infected with cholera vibrios, it usually remains unchanged after 24 hours at 37°C, but on the second day the color becomes noticeably dark in spite of repeated shaking in order to supply oxygen to the hemoglobin. On the third day the blood appears partly lake-colored and on the fourth there is a dry membrane on the surface of the dark and discolored blood. This membrane consists of cholera vibrios. At the same time there is an intense odor of hydrosulphide, which must be considered a product of chemical activity of vibrios in the blood. The fact that hydrosulphide is not produced by other accompanying bacteria is shown by plate cultures of such blood which invariably have proved the purity of culture. Similarly, one cannot assume that H₂S is always formed in the blood by decomposition due to bacteria. Numerous tests with anthrax bacteria, staphylococcus, erysipelas, glanders bacilli, fowl cholera, typhus, etc. failed to establish a trace of H₂S production in the blood. The decomposition of blood by cholera vibrio therefore is a particularly energetic function, although reproduction is more massive than seen in any of the other fission fungi.

This intense reproduction and rapid multiplication takes place in the later stages of blood culture. In the first 24 hours the blood may appear nearly unchanged. Nevertheless, there is some propagation here as demonstrated by microscopic examinations. This initial reproduction may be accelerated by addition to the blood of a few drops of sterilized broth culture of cholera vibrios. The blood thereby becomes more suitable for the growth of cholera vibrios, its disposition is increased; this manifestation probably is related to the ferment production by cholera vibrios. A study made by H. Bitter ^{1/} under my supervision has proved these ferments capable of dissolving these albuminates and to convert them to peptone. Since sterilized cholera broth culture contains these ferments, their addition to blood will promote peptonization of protein substances, by which it is changed into a form suitable for the nourishment of fission fungus cells. Incidentally, this is a case which contradicts the concept of Pasteur's school, according to which native decomposition products of bacteria are always capable of spoiling a medium for the bacterial species in question.

^{1/} On the Ferment Secretion of *Vibrio Koch* and *Vibrio Proteus*. This Archive, Vol. 5, page 241.

These tests therefore prove a certain reproducibility of cholera vibrio in the blood, although initially only in the fibrinated rabbit blood outside of the body. A direct inference about the conditions in living human blood cannot be made therefrom; however, in view of the considerably greater disposition of the human organism for cholera, a certain reproducibility in living human blood cannot be excluded out of hand. If this would be the case, then direct passage of inhaled cholera vibrios into the pulmonary blood capillaries would be feasible.

One should not object that these discussions are baseless because inhalation of cholera vibrios, which are killed by drying, is an impossibility. Chapter 3 offers proof to the contrary, e.g., that cholera vibrios are attached to fog particles and are suspended in the air, from which they may be deposited on gelatin plates in possession of their full viability. The inhalation of such particles laden with vibrios and their penetration into the alveoli would be quite possible. Similarly, the transport of cholera vibrios, once they have reached the blood, through the circulation into the intestine should not meet with any difficulties. I have proved in tests with guinea pigs ^{1/} that this transportation definitely exists for cholera in this species, which is less susceptible than man. It was shown that subcutaneous injection of large quantities of cholera vibrios (5 to 6 cc gelatin pure culture) produces vibrios in the blood and in the intestinal contents, although acute macroscopic changes such as hemorrhages are completely absent. In view of the importance of this finding, proof was furnished with particular care, and I must for this reason uphold this result against the negative results of Wyssokowitsch. The conditions in such tests are much more complicated than most people imagine and for this reason the quantitative measures must be followed very exactly, especially the dosage of the toxin, that is, of decomposition products of bacteria which are instilled at the same time; otherwise the results can never agree. Hueppe ^{2/} has recently reported results of intraperitoneal instillation of cholera vibrios which agree with the aforesaid and also prove the passage of vibrios into the intestinal lumen without the intercession of visible hemorrhages.

Thus the intestinal localization of cholera can be explained by modes other than the most obvious and simple ingestion of the pathogen through the stomach. I do not deny the great probability of the last concept, but I believe that all approaches should be held open as long as the difference between the pathogenic-bacteriological knowledge and epidemiological experience remains as great as is undoubtedly the case today with respect to cholera.

^{1/} This Archive, Vol. 3, page 401-402.

^{2/} Progress in the Knowledge of the Causes of Asiatic Cholera. Berliner Klin. Wochenschrift No. 11. 1887. Page 185.

Illustrations of Plate 1.

- Figure 1. Piece of lung from inhalation of anthrax spores bedded in nutrient gelatin (plate culture), with proliferating anthrax colonies formed from inhaled spores. Magnification 25 times.
- Figure 2. Pulmonary section of mouse killed with chloroform 20 hours after inhalation of anthrax spores. Initial stage of a commencing infectious process. The inhaled spores have germinated and have grown into small groups of rods which are situated partly on, partly between the alveolar epithelium. Magnification 700 times.
- Figure 3. Pulmonary section of the guinea pig which died from inhalation of anthrax rods: anthrax pneumonia. The alveoli are filled with fibrous exudate rich in cells; there are numerous anthrax rods and threads in-midst these exudated masses, while the blood passages are usually quite free of them. Magnification 600 times.
- Figure 4. a.b. Pulmonary capillaries containing copious anthrax bacilli from a section of a guinea pig sacrificed with chloroform 23½ hours after inhalation of anthrax spores. This preparation corresponds to the second stage of lung infection; it shows that the passage of anthrax bacilli into the pulmonary circulation has taken place after 23½ hours. Magnification 700 times.