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Signatur: as/b1/074
DOI: 10.25646/8200
Transkription: Heide Tröllmich

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„Experimental Contribution to the Etiology of Infectious Diseases with special reference to the Doctrine of Contagium vivum." By E. Klein, M.D., F.R.S. Received February 4, 1878

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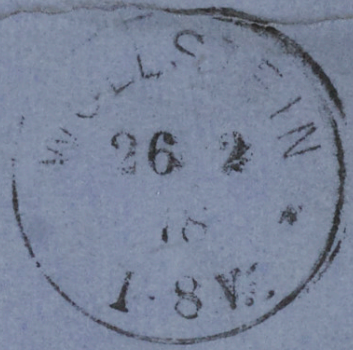

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. "Experimental Contribution to the Etiology of Infectious Diseases with special reference to the Doctrine of Contagium vivum." By E. KLEIN, M.D., F.R.S. Received February 4, 1878.

The present communication has for its object to bring before the Royal Society the results of an experimental inquiry * into the etiology of an infectious disease of the pig, known as Hog Plague, Mal Rouge, Red Soldier, Malignant Erysipelas, or also Typhoid Fever of the Pig. There are English and continental writers who describe the disease as Anthrax or Splenic Fever of the Pig. I shall show, however, conclusively in my report to the medical officer of the Local Government Board, that it is neither typhoid fever nor anthrax, but is an infectious disease of its own kind, which I propose to call "Infectious Pneumo-Enteritis of the Pig" (*Pneumo-enteritis contagiosa*).

Like other infectious diseases, the "Pneumo-Enteritis" possesses an incubation period, followed by constitutional disturbance and certain anatomical changes. These latter are invariably affections of the lung, of the intestine, and of the lymphatic glands, not only of those of the organs of respiration and alimentation, but also those of the inguinal and lumbar regions. In the lung the changes are those known to pathologists as lobular pneumonia. In the alimentary canal the mucous membrane of the large intestine is chiefly affected, being the seat of smaller or larger ulcerations. There is generally also inflammation of the serous membranes, especially the peritoneum, leading to an exudation of lymph into the serous cavity. The skin is occasionally affected with greater or smaller red patches.

There are hæmorrhagic patches to be found in the lung and serous membranes, the endocard, and the muscle of the heart, the mucous membrane of the intestine (especially duodenum and large intestine), the tongue, and occasionally also the liver and spleen, only seldom in the skin and kidney.

In anatomical respects, therefore, the Pneumo-Enteritis bears undoubtedly a great resemblance to anthrax or splenic fever. There exists, however, a marked difference between the two diseases in the incubation period, the general pathology,† and especially in the anatomical character of the spleen and blood. In splenic fever we find the spleen invariably enlarged, being the principal organ of the affection, whereas in pneumo-enteritis it is only occasionally changed. And, likewise, the blood presents entirely different characters in the two diseases; in pneumo-enteritis it is not different in any marked degree from normal blood, whereas in splenic fever it is of dark colour—laky, and does not coagulate at all, or only imperfectly so. Besides, the blood in splenic fever contains the now famous Barillus anthracis, and hence its conspicuous infectious property, whereas in pneumo-enteritis the fresh blood does not, as a rule, contain any foreign matter, and in most instances does not possess any infectious property.

Another disease with which pneumo-enteritis bears a great resemblance on account of certain anatomical characters, viz., inflammation of serous membranes, lung, intestine, and lymphatic glands, hæmorrhage in lung, serous membranes, endocard, muscle of heart, intestinal mucous membrane, and other organs—is specific septicæmia.‡

The resemblance, however, is not greater than to splenic fever, although the differences are not less well defined. Besides others, there is this great distinction, that in pneumo-enteritis the contagion spreads by simple cohabitation, and through the air, which it never does in septicæmia, as in this the virus always requires a broken surface through which to enter a healthy individual. Pneumo-enteritis, occasionally described as malignant erysipelas (mal rouge, red soldier), but this is in so far inadmissible, as the affection of the skin in the former is a very inconstant symptom, and in milder forms of the disease is invariably absent. More recently the pneumo-enteritis has been regarded as typhoid fever of the pig. From a purely anatomical point of view, the resemblance between real, *i.e.*, human, typhoid fever and pneumo-enteritis is very slight indeed, so slight, in fact, that to mention it requires a total oversight of some of the most prominent symptoms, *e.g.*, inflammation of lung and serous membranes, enlargement of inguinal, lumbar, and bronchial lymphatic glands, hæmorrhages in endocard and muscle of the heart in pneumo-enteritis, on the one hand, and swelling and ulceration of the lymphatic glands of the small intestine, swelling and inflammation of spleen in real typhoid fever, on the other hand. The resemblance seems to be limited solely to the fact that in both diseases there occurs ulceration in the intestine. But the distribution, the nature, and the development of these ulcerations is totally different in the two diseases.

Having said thus much as a prefatory explanation, I proceed to state the results of the experiments.§

The experiments refer to the following series:—

1. Experiments showing that the fresh blood of diseased animals does not, as a rule, contain the virus, as it fails to produce the disease when introduced into a healthy animal.

Four animals were inoculated (at different times) with fresh blood of diseased animals. They remained healthy. When subsequently inoculated with virus-containing matter, they became smitten with the disease.

In a fifth instance, however, fresh blood did produce infection. [And this same blood proved active after having been kept sealed up in a capillary tube for several weeks.] This blood was obtained from a very severe case with copious peritoneal exudation; in which were found peculiar abnormally large coarsely granular cells; the same cells were also present in the blood; so that it appears probable that the blood became charged by absorption, during life, with matter from the peritoneal exudation. This latter always contains the virus in an active state.

2. Experiments showing that fluid as well as solid lymph of the diseased peritone contains the virus in a very active state.

Six successful inoculations with fluid peritoneal exudation.

There is no difference of activity to be noticed between fresh exudation and one that had been kept sealed up in a capillary tube for several weeks.

Solid lymph obtained from the peritoneal cavity of diseased animals, having been dried at a temperature of about 38° C., proves very active.

3. Experiments showing that parts of the diseased lung, ulcerated intestine, and also diseased spleen, contains the virus in an active state. Diseased parts of lung or intestine, that were dried at a temperature of about 38° C., retain their virulence unaltered.

In all cases of pneumo-enteritis the trachea as well as the bronchi contain frothy blood-containing mucous matter, possessed of infectious property. It must be, therefore, supposed that the breath of a diseased animal is charged with the poison. On account of the diseased state of the intestine also, the dung is to be regarded as infectious.

4. Experiments showing that infection is produced by cohabitation with a diseased animal, or by keeping healthy animals in a place whence a diseased animal had been removed.

5. Several experiments were made to see whether feeding healthy animals on matter obtained from the diseased organs (intestinal ulcers especially) produces the disease. The experiment was always attended with success, if a lesion-abrasion existed in the mucous membrane of the mouth or pharynx; this was usually the case when the matter had to be introduced into the mouth while the animal was being held by assistants.

There were, however, two cases which appear to prove that the disease cannot be produced by simple feeding.

This was, unfortunately, at a time when I was not acquainted, yet, with the fact that in many animals the disease is of so mild a form that it can hardly be recognized in the living. I have not made any post-mortem examination of those two animals.

But since then I have made two other experiments, in which the virus was brought directly into the stomach, by means of an india-rubber tube introduced per fauces and œsophagum. In both these instances the animals became diseased and their intestines were most conspicuously affected.

From the last three series of experiments, we may conclude that the principal mode by which contagion of pneumo-enteritis is carried out, is through the instrumentality of the air and the food.

6. This series comprises experiments to prove that the virus can be cultivated artificially, *i.e.*, outside the body of an animal; in the case of splenic fever it has been successfully done by Dr. Koch.

The experiments are seven in number, (*a*), two refer to cultivations started with fluid peritoneal exudation; (*b*), in the five others the virus had been obtained by cultivation of dried lymph of the peritoneum of an animal suffering from the disease.

(*a.*) The cultivation of the virus for the first two cases was carried out thus:

Fluid peritoneal exudation of a diseased animal had been collected and sealed up on November 6, in a capillary glass tube. On the following day there was present a small clot due to coagulation. A minute speck of this clot was removed with the point of a clean needle, and with it was inoculated a drop of fresh aqueous humour of a healthy rabbit. This drop had been placed on a thin covering-glass, which, after the inoculation, is inverted over a small "cell," made by fixing a glass-ring* on an ordinary glass-slide. The covering-glass is fastened on the glass-ring by means of a thin layer of pure olive-oil. The preparation was then kept in the incubator for twenty-four hours at a temperature of 32–33° C. After this time it was used to inoculate a new drop of humor aqueous in a similar manner as the one just described. We will call this the second generation.

This new specimen was placed in the incubator and kept there at a temperature of 32–33° C., for further twenty-four hours. In the same manner a third generation was started by inoculating a fresh drop of humour aqueous. After having been kept in the incubator for several days it was used to inoculate two animals at different times. Both animals became smitten with the disease.

(*b.*) The other five experiments were carried out with virus cultivated from solid lymph of the peritoneum of a diseased animal. The lymph had been dried at 38° C (See Series 2). A small particle of dried lymph is crushed into fine powder. With a granule of this a drop of fresh humor aqueous is inoculated in the same manner as above described,—First generation.

* This being part of a larger research carried out for the medical officer of the Local Government Board, ~~with whose permission the present communication is made~~.

† In splenic fever the period of incubation ranges from between a few hours to several days, in pneumo-enteritis it varies from two to five days and more. Splenic fever is easily transmissible to man and the domestic animals, whereas the transmissibility of the pneumo-enteritis is much more limited. Hitherto I have succeeded in communicating it to rabbits, guinea-pigs and mice, although only with difficulty.

‡ Specific septicæmia as distinct from septic-infection. See Dr. Burdon-Saunderson's lectures at the University of London, 1877.

§ In all my experiments of inoculation the materies morbi was used in minimal doses, *i.e.*, a drop of fluid matter, or in the case of solids a particle of less than the size of a pin's head. In both cases the materies morbi was diluted or suspended respectively in a few minims of boiled saline solution of ¾ per cent. in order to increase its bulk and thus to facilitate its introduction. The inoculation was invariably carried out by injection into the subcutaneous tissue by means of a fine cannula of a hypodermic syringe, necessary care being taken that this had been previously thoroughly cleaned and disinfected. After and before inoculation the animals have always been kept isolated and in clean and disinfected places. In order to insure reliable results (viz., that the disease in a particular case was really a consequence of the inoculation and not of infection through other sources) care was taken that those who attended the isolated animals were not the carriers of infection.

* The glass ring I used is 0·5 to 2 millimètres high, about 2 mm. thick, and about 18 mm. wide. If the preparation is to be observed on the hot-stage of the microscope, instead of the ordinary glass-slide, one of only 0·5 mm. thickness is chosen in order to bring the preparation more rapidly up to the desired temperature.

After having been kept in the incubator for two or three days at a temperature of 32–33° C it is used to inoculate a second generation, care being taken to use a trace only of the fluid part and not to come in direct contact with the original granule, which may be still discerned in the preparation.

The specimen representing the second generation is kept in the incubator for a day or two. It is then used to inoculate a fresh preparation.—Third generation. And, finally, this is used for establishing a fourth generation. After having been kept in the incubator, a part of it is used for inoculating *two* animals, the inoculation being carried out at different times.

Both these animals become smitten with the disease. Another portion of this fourth generation was used to start a fifth generation, then a sixth, a seventh, and an eighth generation. With this three animals were inoculated at different times. All three animals became diseased in due time.

In order to correctly interpret the results of this last (6th) series of experiments, it is important to mention that inoculation with dried lymph diluted far less than would correspond to the third generation in the last-named experiments, is followed by a negative result.

The microscopic examination of the cultivated liquids proves that these are the seat of the growth and development of a kind of bacterium, which has all the characters of bacillus subtilis (Cohn). The bacillus in our case is a very fine and delicate rod, thinner than both that described by Professor Cohn in hay-infusion, and the bacillus anthracis so thoroughly investigated by Dr. Koch.

Our bacillus differs also in other respects from that of bacillus anthracis, inasmuch as it possesse a moving stage, the bacillus anthracis described by Dr. Koch as non-moving. Like bacillus subtilis of hay and bacillus anthracis, our bacillus grows under favourable conditions into long leptothrix-like filaments, which occasionally form more or less complex convolutions.

In these filaments highly refractive spores make their appearance. These become free after the disintegration of the original filamentous matrix. The fully developed spores of our bacillus differ from those of hay-bacillus and anthrax-bacillus by being more distinctly cylindrical and much smaller.* According to Professor Cohn (Beiträge zur Biologie der Pflauzen II, 2, 1876, p. 264) the long diameter of the spores of bacillus of hay and also of anthrax—for both are identical in morphological respects (l.c., p. 275)—amounts to 0·0015—0·0022 mm. or $\frac{15 \text{ to } 22}{2500}$ of an inch, whereas the spores of our bacillus are little less 0·0005 mm. or $\frac{1}{2000}$ of an inch in their long diameter.† At first I misinterpreted the spores, regarding them ns a kind of micrococci, and only after repeated observations have I succeeded in tracing them through their different stages of development.

After many failures—owing to the introduction and development of bacterium termo—I succeeded at last in obtaining, already in the second generation of original virus, a pure crop of bacillus and its spores. With these I started several separate cultivations, in which the germination of the spores into delicate bacillus, the swarming stage, the rapid multiplication by division, their growth into long apparently smooth filaments, and, under sufficient access of air, the formation of the bright cylindrical spores could be distinctly traced.† (No other organisms appeared in these cultivations.) These were again used to inoculate other preparations of aqueous humor, and so on, until I succeeded in obtaining considerable quantities of liquid, containing only bacillus and its spores. The last-named animals were infected with liquid of this kind.

Seeing that splenic fever, pneumo-enteritis, and specific septicæmia possess a great affinity in anatomical respects, and seeing that in splenic fever and pneumo-enteritis the materies morbi is a definite species of bacillus—the difference of species being sufficiently great to account for the differences in the two diseases—we may with some probability expect that also the third of the group, viz., specific septicæmia, is due to a bacillus. This, however, remains to be demonstrated. It seems, finally, justifiable to speculate whether or not we have in these three varieties of disease a "a variation of species" in the sense of the evolution theory.

* In the figures accompanying Mr. Koch's paper on bacillus anthracis (Beitr. Z. Biologie d. Pflauzen ii, 2, 1876) the spores are represented in many places as more or less spherical in shape.

† In convolutions of filaments the outlines of these latter become gradually lost after the spores are formed. The spores appear now to be embedded in a transparent gelatinous matrix. At the edges of such masses or where they are in a sufficiently thin layer, the linear arrangement of the spores can be still recognised. But there is undoubtedly a transparent jelly present in these masses forming the ground substance for the spores and fibres. Professor Cohn mentions (l. c., p. 263) a similar jelly in convolution of hay-bacillus. I entirely differ from Dr. Koch with regard to the mode of germination of the spores of bacillus. Koch states (l. c., p. 289, and also in his latest paper on Bacteria in Biol. d. pfl. 2 Bd. 3 Heft.), that it is not the highly refractive spore which directly produces the bacillus, but that the hyaline gelatinous envelope surrounding each spore elongates so as to form the bacillus, while the bright spore-matter itself gradually diminishes and finally disappears. From *à priori* reasons it is impossible to assume that this can be so, viz., that the gelatinous envelope should grow into the bacillus; for Cohn proved beyond doubt that in the case of hay-bacillus the spores germinate even after having been exposed to boiling heat. Surely this gelatinous envelope, if living protoplasm, must become, under these conditions, deprived of its germinating power. Direct observation proves that in my case the spores passes another membrane within that gelatinous envelope and during germination this inner membrane is broken at one pole and the contents of the spore protrudes and grows out as the bacillus. This is also in accordance with the observations of Professor Cohn, for this authority states (l. c., p. 265) "Die sporen schwollen etwas an und trieben an einem Ende einen kurzen Keimschlauch."