

STUDIES IN DIPHTHERIA.

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A CONTRIBUTION TO THE PATHOLOGY OF EXPERIMENTAL DIPHTHERIA.

BY A. C. ABBOTT, M. D., and A. A. GHRISKEY, M. D.

DIPHTHERIA WITH BRONCHO-PNEUMONIA.

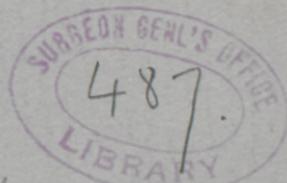
BY SIMON FLEXNER, M. D.

ACUTE ULCERATIVE ENDOCARDITIS DUE TO THE BACILLUS DIPHTHERIÆ.

BY W. T. HOWARD, JR., M. D.

REMARKS.

BY W. H. WELCH, M. D.



presented by A. C. Abbott, M. D.

A CONTRIBUTION TO THE PATHOLOGY OF EXPERIMENTAL DIPHThERIA.

WITH SPECIAL REFERENCE TO THE APPEARANCE OF SECONDARY FOCI
IN THE INTERNAL ORGANS.

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AND

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(From the *Laboratory of Hygiene of the University of Pennsylvania*. Read before the *Johns Hopkins Hospital Medical Society*, March 20th, 1893.)

In the course of our studies upon the alterations produced by the bacillus diphtheriæ in the tissues of susceptible animals we have encountered upon several occasions a condition that, so far as we are aware, has either escaped notice or has not been mentioned by other writers upon this subject. In addition to the macroscopic alterations commonly observed in the experimental form of this disease, and the microscopic lesions described by Oertel¹ in the tissues of human beings dead of diphtheria, and by Welch and Flexner² in the tissues of animals that have succumbed to inoculation with the diphtheria virus, we have detected in seven autopsies a condition of the omentum that we propose to describe in this contribution.

The animals upon which the experiments were performed were guinea-pigs, and the cultures employed were but a few generations removed from undoubted cases of primary diphtheria.

¹Oertel: "Die Pathogenese der epidemischen Diphtherie. Nach ihrer histologischen Begründung." Mit Atlas. Vogel, Leipzig, 1887.

²Welch and Flexner: "The Histological Changes in Experimental Diphtheria." *Johns Hopkins Hospital Bulletin*, No. 15, August, 1891. Also, "The Histological Lesions produced by the Toxalbumen of Diphtheria," *Ibid.*, No. 20, March, 1892.



The special lesions, which form the subject of this paper, consist in minute, yellowish, lens-shaped foci located in the omentum, usually between its peritoneal layers, and usually circumscribed in outline. When visible to the naked eye they are rarely larger than the eye of a small cambric needle, and of about the same shape. They are occasionally larger than this, but more commonly smaller, being frequently so small as to be detected only by microscopic examination of sections of the hardened and embedded omentum. As a rule the omentum containing these bodies is somewhat congested, and often presents a shriveled appearance at its free margin. It is in this free margin that the foci are most commonly to be found. Upon microscopic examination these nodules are seen to be sometimes very sharply circumscribed, while again they are slightly less regular in outline. At times they may be seen lying between folds of the peritoneum (Fig. 1), while again they will be limited by the walls of lymphatic spaces. Upon closer examination they are seen to consist of polynuclear leucocytes and those having the horseshoe-shaped nucleus, closely packed together. We have rarely detected any of the fixed tissue elements within these nodules. The majority of the leucocytes composing them contained bacteria which in their morphological and staining peculiarities were identical with the bacillus diphtheriæ.

In one instance in which there was an unusually large nodule we obtained from it the virulent diphtheria bacillus in pure culture; in the majority of cases, however, the nodules were so small and the danger of error during manipulation so great that we decided to rely upon microscopic examination for the identification of the bacteria within them.

The histological structure of these nodules is simple, and there is but little to describe beyond that which has already been mentioned, namely, that so far as we have been able to make out they are nothing more than masses of leucocytes, many of which are in the phagocytic condition, crowded together within the lymph channels of the omentum. At times they may be seen lying directly beneath one of the layers of the peritoneum, while again they may occupy lymph canals between the two layers where these are in juxtaposition. As a rule there is little or no reaction of the tissues about them, and they appear only as if the lymphatic space in which they are located had become plugged up by them.

In one case in which an unusually small amount of material had been injected into the animal, only two of these foci could be found, and these only upon microscopic examination of sections of the omentum. They were characteristic in their histological structure, but most careful search failed to reveal the presence of bacteria in either of them.

In a number of other cases that we have examined the omentum has been found congested and often diffusely infiltrated with leucocytes along and about the course of the vessels, but in none of these instances could we detect the circumscribed accumulations of leucocytes to which we are referring. It was also impossible for us to detect micro-organisms of any character by microscopic examination. In six cases in which the bacilli were present in the omentum they were always within the circumscribed nodules of leucocytes, either free or within the body of cells. In the majority of instances, so far as we can state from careful examination of the sections, they were within the bodies of phagocytes. In one case in which there was a slight peritonitis we found a few endothelial cells containing bacilli.

In our search for these nodules, after first having detected them, our attention was frequently attracted to small, whitish points in the omentum, which often led us to believe that the foci of leucocytes were present, but which, upon microscopic examination, proved to be minute lymphatic bodies, evidently rendered conspicuous through the action of the diphtheritic poison, an enlargement analogous to that seen in the grosser lymphatic structures when under the influence of this poison. In some of these lymphatic structures leucocytes could be detected, but in no instance have we found bacilli within them. In one instance a karyokinetic figure was observed.

The first three cases in which our attention had been attracted to these nodules were animals that had been inoculated subcutaneously. The inoculations were made in the tissues of the abdominal wall a little to the right of the median line and about midway between the free margin of the ribs and the inguinal region. In two of these animals fluid cultures were employed, while in the third a portion of a solid culture on Löffler's blood-serum mixture was used.

In our efforts to cause the reappearance of the omental nodules various methods of inoculation were employed. It was plain that the passage of the bacilli from the seat of

inoculation in the abdominal walls to the omentum where they were found was by means of the lymph canals, the course being in all probability by way of the inguinal and retro-peritoneal lymph glands, though microscopic examination of both of these structures failed to reveal the presence of bacilli within them. This opinion was strengthened by an experiment made by injecting into the same locality of another animal a small quantity of sterilized India ink. The animal was killed after 48 hours, and not only the inguinal and retro-peritoneal glands were found to be pigmented, but the omentum as well, though in the case of the latter the pigmentation was not circumscribed and was not present in nodules similar to those seen in the cases in which cultures of the bacillus diphtheriæ had been used.

We tried superficial and deep injections, sometimes immediately beneath the skin, and again as nearly between the muscular layers and the peritoneum as possible without entering the latter, but no constancy in our results could be obtained, and it is impossible for us to say upon what factor the presence of the foci found in the omenta of these three cases depended. The tissues at the site of inoculation were examined microscopically, but nothing was found to aid us. There were several features, however, in addition to conditions mentioned by other observers,¹ that may be interesting to note, viz.: In a few sections made directly through the point at which the bacilli had been deposited we found, in addition to the hyaline condition of the muscle fibres, that there could, here and there, be detected a penetration of these degenerated tissues by the bacteria that were lodged in clumps in the lymphatic spaces adjacent to them—a condition that we have endeavored to represent in Plates 4, 5 and 6, and which is apparently identical with the condition of the muscle fibres described by Klein,² at the seat of inoculation of two cows into which bouillon cultures of the bacillus diphtheriæ had been introduced. We have failed, however, to detect the mycelial-like threads to which he makes reference, and have found only the long, irregularly staining forms that are common to

¹ See Welch and Abbott: "The Etiology of Diphtheria," Johns Hopkins Hospital Bulletin, No. 11, February and March, 1891, p. 29.

² Klein: The Etiology of Diphtheria. Nineteenth Annual Report of the Local Government Board, 1889-90. Supplemental Report of the Medical Officer, pages 173 and 174.

blood-serum cultures of this organism. Sometimes the penetration would occur through the side of a fibre, as seen in cross section (Fig. 4), while again the bacilli grew in from one end (Fig. 5), or from both ends and the side (Fig. 6). It was also a common occurrence to find degenerated muscle fibres into which leucocytes had wandered. In the main, though, the local changes were the same as those given by others who have studied the process in detail.

After failure to produce the omental foci in five out of eight cases, we abandoned the method of subcutaneous inoculation for one that we thought more promising in its outlook. Bearing in mind the fact that it is possible to inject in a fairly perfect way the lymphatic system of small animals through the testicle, this point was chosen for subsequent inoculations. Four animals were inoculated with either bouillon cultures or suspensions of the bacilli in physiological salt solution, by direct injection into the testicles. The results of these injections were satisfactory in each case; two of the animals that had received bouillon cultures, the one 0.6 cc., the other 0.7 cc., presented at autopsy most exquisite omental foci, in all respects identical with those present in the first three animals studied, except they were more numerous; while the remaining two animals, the one of which had received 0.4 cc. and the other 0.5 cc. of a salt solution suspension of the bacilli, presented the foci, but fewer in number and smaller in size. Those from the animal that had received 0.5 cc. were characteristic in histological structure, and contained bacilli which under Gram's method of staining showed the characteristic morphology of the bacillus diphtheriæ. The remaining animal had also foci in its omentum which were histologically identical with those seen in the other animals, but in which no bacteria could be detected. We cannot account for the absence of bacilli from the foci of leucocytes in this case.

A point of particular interest in connection with the cases in which the injections had been made into the testicle was the diminution of œdema of the superficial tissues and its increase in the tissues of the abdominal cavity. In two of these cases straw-colored fluid was not only found free in the peritoneal cavity, but the small intestine was practically filled with fluid and its walls were intensely œdematous; and in all four of these cases the œdema of the subcutaneous tissues over the point of inoculation was markedly diminished. In these

cases also the retro-peritoneal lymph glands, particularly those on the side inoculated, show a marked reaction, and we felt reasonably sure of detecting the bacilli within them; but most careful microscopic search failed to reveal them in these tissues, though the omentum may have contained numerous foci of leucocytes and large numbers of bacilli. It is possible, however, that by culture methods we might have obtained them from these tissues, but we considered the possibility of error under these circumstances so great that only microscopic examination was thought safe.

The method of examination to which we have given the preference consists in staining the tissues lightly in a watery solution of Bismarck brown, and after carefully washing them out in alcohol to subject them to the method of Gram. If bacteria are present they appear in very striking contrast upon the brown background (see Fig. 3). If the Gram method is used alone or with eosin, as we have tried it, the possibility of error is great. The chromatin of the nuclei of the tissue cells frequently takes on and retains the violet stain, and portions of the nuclear beam-work may appear so like the bacillus diphtheriæ or clumps of this organism as to make the differentiation by this method very difficult. By the method that we have employed the nuclear network stains brown, and does not lose this color when subsequently treated with the violet dye. A very good idea of the contents of these nodules can also be formed by crushing one upon a cover-slip and manipulating it in the way commonly employed in making cover-slip preparations. A portion of one of the nodules treated in this way and stained in Löffler's blue is depicted in Fig. 2.

Until recently the opinion in regard to the relation of the diphtheria bacilli to the organism in which they are located has been, that they remained at the point at which they were found or had been deposited, but recently evidence has been presented that will cause some modification of this view.

Frosch,¹ in a series of bacteriological studies upon the cadavers of individuals dead of diphtheria, has detected the bacilli in the internal organs of ten out of fifteen cases examined by him at autopsy. He found them in the brain, lungs, liver, spleen, kidneys, cervical and bronchial glands,

¹Frosch: Verbreitung des Diphtherie-bacillus im Körper des Menschen. Zeit. f. Hygiene u. Infectiouskrankheiten, 1893, Band XIII, p. 49-52.

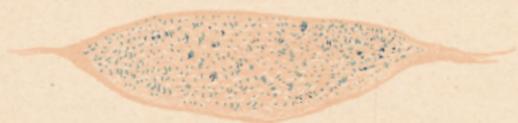


Fig. 1

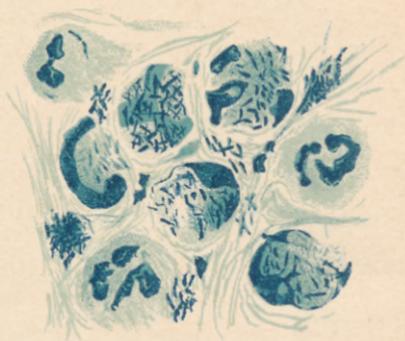


Fig. 2

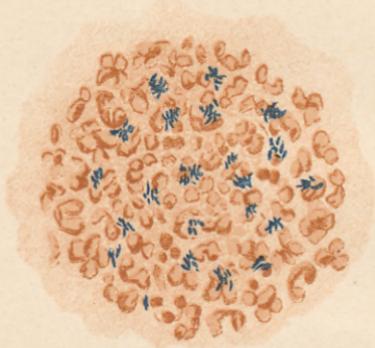


Fig. 3

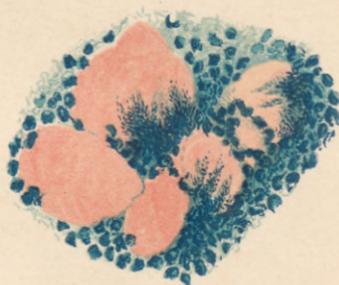


Fig. 4



Fig. 5



Fig. 6

blood, and in the pericardial and pleural fluids. They were least frequently present in the liver; they were regularly to be found in pneumonic nodules, in the spleen, and in the bronchial and cervical lymph glands. In only one of the ten cases were they missed from the blood of the heart.

Through which channels the bacilli gain access to the internal organs in human diphtheria it is, in the present state of our knowledge, impossible to say, but that it is through the lymphatics, and largely through the aid of the wandering phagocytic cells, that they appear as secondary depositions in the experimental forms of the disease, we think our observations just cited leave but little doubt.

We have had no evidence in our cases to lead us to suppose that the migration occurs through the vascular system. On the contrary, in those omenta that were congested and more or less diffusely infiltrated with leucocytes, particularly along the course of the vessels, we have not succeeded in detecting the bacilli either free or in phagocytes in a single case, whereas, on the other hand, in the cases in which they did appear in the omentum it was, as stated, only where the phagocytes were densely packed within the lumen of a lymph canal, or in connective tissue spaces between or beneath the peritoneum.

DESCRIPTION OF PLATE.

Fig. 1. Section through focus of leucocytes located in the omentum between the layers of the peritoneum. Leitz, obj. 3, ocular I, tube 160 mm.

Fig. 2. Contents of one of the foci dried upon a cover-slip and stained with Löffler's blue. Leitz, o. i. $\frac{1}{2}$, ocular III, tube 160 mm.

Fig. 3. Section of one of the foci that had been hardened in absolute alcohol, stained with Bismarck brown and subsequently by Gram's method. Leitz, o. i. $\frac{1}{2}$, ocular III, tube 160 mm.

Figs. 4, 5, 6. Hyaline muscle fibres from seat of inoculation, showing penetration of bacilli into them. Stained with eosin and methylene blue. Leitz, obj. 7, ocular III, tube 160 mm.

DIPHTHERIA WITH BRONCHO-PNEUMONIA.

BY SIMON FLEXNER, M. D., *Associate in Pathology, Johns Hopkins University and Hospital.*

The contribution which I have to make to this subject is based upon the examination of two cases of diphtheria in human beings in which the smaller bronchi were invaded by the diphtheria bacillus. The fact that the diphtheria bacilli are taken up, in these examples of Dr. Abbott, by leucocytes is borne out also by the results obtained in at least one of the cases to which I refer. This case has been presented to the society before, so that nothing at this time need be said concerning the gross anatomical features. As I mentioned then, cultures made from the lung proved the diphtheria bacillus to be one of the micro-organisms present. Our idea at that time was that the organisms were not actually derived from the parenchyma of the lungs, but it was probable that we had entered a small bronchus which we knew had been invaded by the diphtheria bacilli, since a typical diphtheritic membrane could be made out in all the smaller bronchi. We realized that it would require a histological examination of the tissues to prove whether or not the diphtheria bacilli were actually present in the alveoli of the lung.

In the second case, the diphtheria bacilli were not found in cultures made from the lung. There were broncho-pneumonic areas in the lungs of both cases, and in these the micrococcus lanceolatus was obtained from the nodules.

The publication to which Dr. Abbott has referred made us still more interested in determining whether or not the diphtheria bacilli were present in these areas in the lungs. Up to the present time, on account of circumstances over which I have had no control, the complete study of these two cases has been impossible. Still I have been able to cut sections from various parts of the lung, from the areas of broncho-pneumonia and elsewhere, and from the larger bronchi near

the root of the lung and the trachea, and I can say this much that the diphtheria bacilli are present in the smaller bronchi which are found in these areas of broncho-pneumonia, and that they are there not alone in the membrane but also in the exudate which fills the bronchi. This exudate consists almost entirely of polynuclear leucocytes, and the protoplasm of these leucocytes is crowded with diphtheria bacilli. Some time was spent in searching for bacilli in the broncho-pneumonic areas. In two instances I felt quite sure that I had found isolated diphtheria bacilli in them, but they were not there in large numbers. It may prove, on further examination, that they are more common. They are certainly much less numerous than the micrococci lanceolati, which are present in considerable numbers.

With regard to the phagocytic action of the leucocytes in reference to the bacillus diphtheriæ, it was observed by Dr. Welch and myself that not infrequently when the bacilli were injected beneath the skin the leucocytes took up some of them. In some instances these leucocytes preserved their normal appearance, while in others they appeared to be injured, the nucleus being destroyed or fragmented and the cells dead.

I have made some experiments relating to the production of a pseudo-membrane in which were used dead diphtheria bacilli alone, and also dead bacilli which had been combined with the filtrate from a bouillon culture of the diphtheria bacillus. I found that if I injected the dead diphtheria bacilli alone a small nodule formed at the site of injection, which, after a day or two, shrunk in size and finally disappeared. But if the dead diphtheria bacilli were injected along with a filtrate from a bouillon culture, the animal would die in consequence of the action of the diphtheritic virus. The dead bacilli gave rise then to a nodule, and on cutting sections of this nodule we found that the dead bacilli had been taken up by the leucocytes and presented much the same appearance under the microscope as the specimens which Dr. Abbott exhibits.

ACUTE ULCERATIVE ENDOCARDITIS DUE TO THE BACILLUS DIPHThERiÆ.

BY W. T. HOWARD, JR., M. D.

The history in brief was that of a man, a Russian Pole, æt. 44 years, married, a laborer, who was admitted to St. Joseph's Hospital, November 7th, 1892, in the service of Dr. F. A. Warner. He complained of weakness, pain in the head, and diarrhœa. His previous history had been good. His present illness began on November 1st, with a chill, high fever, pain in the head, abdomen and limbs; nausea, vomiting and diarrhœa. The man remained in this state for seventeen days; his temperature running an irregular course, his pulse at first was of good volume and under 90 beats per minute, but towards the last was very weak and rapid. He died in collapse.

His heart, which I show here, is larger than normal. The myocardium is firm and rather pale. The coronary arteries appear normal. On the upper or auricular aspect of the mitral valve there is situated a mottled red and white, irregular, granular-looking thrombus mass. This mass begins just below the base of the valve and completely covers both segments. Its greatest thickness is at the free border of the valve. The mass is everywhere intimately adherent to the underlying valve tissue, and varies in thickness from 0.2 to 1 cm. On removing portions of it with the finger the endocardium is found to be rough, ulcerated and hemorrhagic. On the under or ventricular surface of the posterior segment there are several smaller points of ulceration, which are in places covered with the same fibrinous material seen on the upper surface. The lower surface of the anterior segment is free from ulceration. When the surfaces of the thrombus mass are brought together the auriculo-ventricular opening is almost completely closed. There are a few small vegetations

on the ventricular surface of the aortic valve. The parietal endocardium is unchanged and the other valves are normal.

The liver was large and very much congested.

The spleen was very large, soft, and nearly the whole organ was the seat of an infarction. In the larger branches of the splenic artery there were white and red thrombus masses.

Both kidneys showed acute nephritis and were the seat of numerous infarctions.

Cover-slip preparations made from the thrombus mass on the mitral valve, and from the spleen and kidneys, all showed the presence of a bacillus with all the morphological characters of the bacillus diphtheriæ. This bacillus was the only organism present. Cultures were made on glycerine agar from the mitral valve, the lungs, the liver, spleen and kidneys.

In the tubes from all these sources were obtained in pure culture numerous colonies identical in appearance with colonies of the bacillus diphtheriæ. On the second day after the autopsy, cultures made from the heart thrombus were inoculated by Dr. Welch and myself into a rabbit and into a guinea pig. Neither of the animals died.

The bacillus has been studied by Dr. Welch, by myself and by several other workers in the laboratory here, and by Dr. Abbott in Philadelphia, and none of us has been able to make out any difference between this bacillus and the bacillus diphtheriæ, either morphologically or by culture methods. The only point of difference is that this bacillus has so far failed to kill animals.

For histological study of the process in the mitral valve I have made sections through the posterior segment, perpendicular to the surface. Beginning at the base of the valve, at its insertion, there is a loss of the endothelial cells of the endocardium. This is the first step in the process. Just above the thrombus mass here there is a large area over which the endothelial cells are lost. Here also there is a considerable local reaction, shown by a proliferation of the fixed cells of the part and by an infiltration of the tissue by a large number of polynuclear leucocytes. Just at the edge and on the surface of the valve there are great numbers of bacilli in the exudation. The bacilli do not penetrate the tissue to any distance, and are not seen deeper than the first two or three rows of cells.

Immediately below this area the thrombus mass commences, and, under the low power, several different appearances are

met with. The mass in sections stained with eosin and methylene-blue is seen to be made up of different layers. On the free surface there is a thick layer composed of myriads of these bacilli; beneath this is a layer of fibrin, very poor in cells. Scattered about in this fibrinous mass are large and small clumps of bacilli. The bacilli here are usually placed in long, thick bands, but may be seen in smaller scattered masses. At the point of junction between the endocardium and the thrombus mass the endothelial cells are entirely lost, and at this place there is a dense line of bacilli. The endothelial cells cannot be made out at all. Beneath the layer of bacilli the muscle tissue of the valve is hyaline.

Very extensive and interesting changes, which we will not go into here, are shown in the kidneys. There are no areas of necrosis seen in the liver, although many bacilli can be made out in the capillaries.

Histological study shows that the bacillus concerned in the lesions present in this case is to a certain extent a pus producer. In certain areas in the mitral valve especially there is shown a considerable amount of local reaction. The hyaline degeneration noted in the muscle tissue of the valve is of particular interest.

The striking points in this case are a malignant endocarditis with general infection caused by a bacillus that is morphologically and by culture methods indistinguishable from the bacillus diphtheriæ.

For those interested I pass around for comparison cultures in various media of the organism found in this case and of the laboratory stock culture of the bacillus diphtheriæ. It will be seen that the two are identical.

Under the microscopes I have placed sections of the mitral valve showing the lesions and the bacilli described.

(This case will be reported in detail later.)

REMARKS BY DR. WELCH.

Dr. Abbott's observations that the bacillus diphtheriæ may produce lesions containing the bacilli at points distant from the seat of inoculation are interesting. It has been generally supposed that in experimental inoculations of guinea-pigs with this micro-organism, the bacilli develop only locally at and near the point of inoculation, and that they do not multiply in the internal organs and tissues distant from the inoculation. We must, in the light of these new observations, modify to some extent this view, but Dr. Abbott's results do not seem to me absolutely discordant with our previous conceptions. He was able to obtain the foci in the omentum containing bacilli after inoculation, both in the abdominal subcutaneous tissue and in the testicle, more regularly after the latter procedure. By the latter method especially the bacilli might pass directly along the lymph-channels to the peritoneum, as it is well known that the abdominal lymphatics can be readily filled with injecting fluid by injecting into the testicle, so that we may suppose that the bacilli are introduced into the peritoneum almost as directly as they are into the subcutaneous tissue by hypodermic inoculation. The same thing might happen, although less easily and frequently, by inoculation into the abdominal wall. I should be inclined therefore to attribute these omental foci to the direct and rapid penetration of the bacilli from the seat of inoculation to the peritoneum, so that the conditions for their production would not be so unlike those for the formation of the lesions near the point of inoculation. This view might be tested by determining whether the omental foci can be produced by inoculations in situations less directly connected by lymphatic channels with the peritoneum, than is the case with the testicle and the abdominal wall.

Dr. Flexner's communication is interesting, as showing the depth to which the bacilli can penetrate into the smallest bronchial tubes. It does not, however, subvert the current opinion that the complicating broncho-pneumonias of diph-

theria are due to infection with other micro-organisms, the streptococcus pyogenes most frequently, occasionally the micrococcus lanceolatus and other bacteria.

I have followed with exceptional interest the study of Dr. Howard's case of ulcerative endocarditis. We found in this case in pure culture and in large number in the valvular vegetations, the spleen and the kidney, a bacillus absolutely indistinguishable from the Klebs-Löffler bacillus of diphtheria in its morphological and cultural properties. The two organisms have been studied most carefully side by side in all sorts of culture media, and no distinction can be found between them. Dr. Howard's bacillus, however, is not pathogenic to guinea-pigs. Does this suffice to separate it as a distinct species from the genuine Klebs-Löffler bacillus? According to the investigations of Roux and Yersin, of Dr. Abbott and others, the genuine bacillus diphtheriæ may occur devoid of pathogenic properties in our animals used for experiment. If then we admit that Dr. Howard's bacillus is in reality the bacillus diphtheriæ, we have the first recorded observation of a case of ulcerative endocarditis due to this organism, and we are reminded of the old name for this affection, diphtheritic endocarditis, a name based, however, on anatomical rather than etiological reasons.

