THE PATHOLOGY OF DIPHTHERIA.¹

BY WILLIAM T. COUNCILMAN, M.D., BOSTON.

IN speaking to you on the pathology of diphtheria it is impossible for me to give any new facts, or even to present the subject in a new way. Diphtheria to-day is probably the best known of any of the infectious diseases. Even in tuberculosis there are more problems to be solved by investigation. No other disease illustrates better how much a knowledge of the etiological factor has served to clear up the obscure points, both in the pathological anatomy and in the clinical features. At present the only obscure points in the pathology of the disease have reference to general pathology, and apply to other diseases as well.

By diphtheria we mean a disease produced by the action of a bacillus with definite morphological and biological properties which distinguish it from other micro-organisms, and which is known as the Klebs-Löffler bacillus. The anatomical lesions of the disease produced by this organism vary so much in their character, situation and extent, that these alone are not sufficient to characterize the disease. Moreover, similar anatomical lesions may be produced by the action of other causes. Usually, in every disease due to a definite cause, we are able to recognize the character of the disease by the character of the anatomical lesions produced by the action of the cause. In tuberculosis, for instance, we find certain alterations of tissue which are due to the action of the tubercle bacillus, and which have the same general character whether found in man or in animals. In other diseases, particularly in those in which the lesions have the general type of inflammation, similar lesions may be produced by a variety of causes. The recognition of the etiological factor thus becomes the only true basis for a scientific nomenclature of disease. It is also of the greatest practical importance for the hygienist and the clinician that the etiology should be made clear. Similar lesions, as we have said, may be produced by a variety of causes. In one case by an organism having little power of infectiousness when transferred to another individual, in another by an organism which under almost all circumstances carries with it the power of infecting other individuals. Treatment and hygienic measures based on a recognition of the cause are more intelligent and more efficacious than when simply directed against the lesions or the clinical symptoms. In diphtheria a special method of treatment has been found which is efficacious only against the conditions produced by the diphtheria bacillus.

In the pathology of an infectious disease we have to consider lesions of various sorts. In the first place, there is some point where the tissues have been invaded by the organisms and where lesions due to their direct action on the tissues will be produced. These are primary lesions of the disease. From this place the organisms may be carried to other parts of the body either along the same surface or by means of the blood or lymphatic circulation, and lesions similar in character may be produced in remote parts. In certain diseases which are distinguished as the toxic infectious diseases, and the best types of which are found in diphtheria and tetanus, certain soluble chemical substances are produced where the micro-organisms

are localized, and these are absorbed and produce various lesions, some of which can only be recognized by the disturbances of function which they produce; others are apparent on careful examination. A third class of lesions may be produced by other organisms than the one causing the disease. These find in the primary lesions conditions favorable to them, and by their action may intensify the primary lesions or effect other parts of the body. The action of these other organisms makes many diseases mixed infections, and in the infectious diseases they play an important rôle. In an advanced case of pulmonary tuberculosis, only a part of the lesions which we find are due to the action of the tubercle bacilli. Many of the inflammatory conditions are due to the action of the pyogenic micrococci which find suitable conditions for their growth in the lesions produced by the tubercle bacilli. In diphtheria the death of the individual is frequently due to the action of these extraneous organisms, against whose action the specific treatment of the disease has no influence.

In diphtheria, in almost all cases, the primary lesions are found in the mucous membrane of the mouth, pharynx or larynx. The name of the disease is derived from the character of the primary lesions. There is an intense inflammation of the part affected, and it becomes covered with a membrane. The macroscopic appearance of this membrane is familiar to you all. Its situation and its character varies. It usually appears first over the tonsils, and from here extends over the adjoining mucous membrane, and may invade the soft palate, the base of the tongue, the pharynx and air-passages. In the air-passages it may extend from the larynx into the trachea, and even into the ramifications of the bronchi. Sometimes the membrane extends in an almost continuous layer over all these parts, sometimes only small isolated patches are seen. Large continuous masses of membrane may be formed by confluence of smaller patches. The color of the membrane, and its connection with underlying tissues, varies. In a well-marked case it has a dirty grayish or brownish color and cannot be easily removed from the surface. When it first appears it is thinner, of a whitish color, and may be removed from the surface. After death its connection with the tissue beneath is less firm, and it may be stripped off in large shreds. The adhesion of the membrane to the tissue beneath varies in different places. In the air-passages it is less adherent than in the pharynx, and not infrequently during life large masses of the membrane may become separated and coughed up.

The epiglottis also shows a varying degree of involvement in the process. There may be only small foci scattered over the surface of this, or the entire epiglottis may be converted into a dense, hard mass covered and infiltrated with fibrin. When the membrane is forcibly removed during life a raw surface remains. The mucous membrane in the vicinity is deeply injected, and there is swelling and edema of all the adjacent tissues.

On microscopic examination of the membrane it is found to consist largely of fibrin, in the meshes of which leucocytes and remains of the epithelial cells are found. The membrane does not consist of a homogeneous mass, but certain differences in structure may be recognized in it. Usually on the surface there is an indistinct granular mass which contains various sorts of bacteria. Beneath this a more regular mesh-

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work of fibrin appears, and below this again we find the fibrin in large, more or less regular meshes. In this mesh-work the character of the fibrin varies. In some cases the filaments are very fine, in others there are broad hyaline-looking masses. The surface beneath the fibrin is uncovered by epithelium. In some cases the epithelium is entirely destroyed, in others here and there, a few cells still adhering will be found. If the section has been made at the edge of the patch the fibrin may be seen extending over the epithelium.

A marked difference will be found in the relation of the membrane to the surface beneath, in the larynx and trachea as compared with the pharynx. In the trachea especially the connection of the membrane with the surface is much looser. It will usually be more or less separated from the surface by the necessary manipulation in hardening and cutting the sections, and a space will be found beneath it. The membrane is sharply separated from underlying tissue by the basement membrane of the epithelium. This basement membrane is swollen and hyaline, and more or less necrosis in the tissue immediately beneath it is seen. The blood-vessels are dilated, surrounded by numbers of leucocytes, and some of them contain masses of fibrin. The walls of many of the more superficial vessels are hyaline. In the pharynx, and particularly over the tonsils, there is a close connection between the membrane and the subjacent tissue. The dense basement membrane which forms the limit of the membrane in the trachea is not found here. There is some formation of fibrin in the loose interstices of the connective tissue, the filaments of which may be continuous with that on the surface. The membrane extends directly into the depressions and crypts of the tissue and into the mouths of the mucous glands. The tissue changes are similar to those described in the trachea. There is the same congestion of the blood-vessels, cellular infiltration, thrombosis and fibrin formation. In the later stages of the disease in both the pharynx and air-passages the membrane may become separated by a cellular exudation beneath it. After death it is softer and more easily removed.

The relation of the Kleb-Löffler bacilli to the membrane is interesting. The bacilli are most easily stained by the same method which stains the fibrin. Immediately on the surface of the membrane great numbers of various bacteria will often be seen. These are principally streptococci and other forms of cocci and bacilli. It is probable that these organisms find a suitable place for their development in the membrane, and to their action may be ascribed the breaking down of the fibrin reticulum to a granular mass. Among these various organisms the Klebs-Löffler bacilli can be recognized. They occur in definite groups, and can easily be distinguished from the other forms. Immediately below the surface the other organisms become less abundant and the groups of the Klebs-Löffler bacilli more numerous. In the lower portion of the membrane where the fibrin net-work is more dense, all of the bacteria are less numerous and often entirely absent. The subjacent tissue, even when intensely inflamed, rarely shows any organisms.

This description holds for the typical cases of diphtheria, but most cases seen at autopsies are not typical. In the majority of cases at the autopsy we do not find an extensive and definite membrane extending over the pharynx and uvula. In many

cases only here and there small patches of fibrin are seen on the swollen dark-red mucous surface. Superficial ulceration and necrosis often mark the place occupied by the membrane during life. In some cases the only membrane seen is on the under surface of the epiglottis. The membrane is often so loose that it may be washed off.

There are various views held as to the formation of the membrane. These views are of importance in diphtheritic processes generally, and do not hold exclusively for diphtheria. The view most generally accepted is that of Weigert, in accordance with which the process consists primarily in a necrosis of the epithelial surface. The exudation from the vessels beneath comes in contact with this necrotic material, coagulates and forms fibrin. In support of this view we know that fibrin is always formed when an exudation comes in contact with necrotic tissue. No matter how the necrosis is produced, whether by chemical or mechanical injury, or by the primary action of bacteria, fibrin is always deposited in and around the necrotic cells. This view of primary necrosis is also held with some modification by Virchow. In his last publication on the subject he defines the diphtheritic process as necrosis of tissue. This does not necessarily lead to the formation of a pseudo-membrane, but when this is formed it is after necrosis has taken place. The epithelium is destroyed and removed, and the fibrinous exudation forms on a surface deprived of epithelium. Another view is that of Wagner, which has been accepted with certain modifications by Recklinghausen, and which is more fully expressed in the work of Smirnow on membranous gastritis. According to the view of Wagner the changes consist in a more or less marked hyperemia and inflammation of the tissue beneath. The membrane is formed in the place of the epithelial layer and is not an exudation from the vessels beneath. It is due to a fibrinous metamorphosis of the epithelial cells themselves. The cells are directly transformed into fibrin, the appearance of a network being given by vacuoles in the centre of the cells, with fibrinous processes proceeding from them. Wagner figures epithelial cells so metamorphosed that the cell is converted into an irregular vacuolated mass, with long processes proceeding from it, giving to the cell an appearance of branching antlers. Recklinghausen has modified this view of Wagner's in that he makes the formation of the diphtheritic membrane a part of the general process of hyaline degeneration. The epithelial cells undergo a hyaline degeneration, the hyaline material is formed in the cells and exuded from them, forming the reticulum. The process also extends into the tissue beneath, in which there is a similar hyaline degeneration of the walls of the vessels and of the tissues. According to Recklinghausen this hyaline degeneration of the tissues is the primary, and the inflammatory processes are superadded to this.

Oertel, in his great work on diphtheria, believes that the formation of the membrane is due to the coagulation of the exudation, and that the epithelial cells themselves take no part in it. The most careful of the recent investigations as to the nature of the diphtheritic membrane is that of Heubner. Heubner investigated the membrane during life by stripping off small pieces which were hardened in various ways and examined. He examined in this way portions of the membrane in various stages of the disease, commencing

on the second day. He found that in the beginning of the disease the membrane was formed in the most superficial layer of the epithelium, and was due to coagulation between the cells. The epithelial cells themselves were not always necrotic and their nuclei could be recognized. With the extension of the membrane formation the lower layers of the epithelium were gradually implicated in the process, and here also coagulation of the exudation coming from the subjacent vessels could be made out. According to Heubner the Klebs-Löffler bacilli are not found in the membrane itself before the third day of its appearance. It would seem most probable, from what we have observed of the membrane formation, that all these views are more or less correct. The membrane is formed both by a coagulation of the exudation and by a fibrinous metamorphosis of the cells themselves; not only of the epithelium, but also of the leucocytes which, in the early stages of the process, make their way from the subjacent tissue into the epithelium. We know that under other conditions from the cells themselves a material can be formed which has the micro-chemical reactions of fibrin.

This membrane formation, as we have said before, is not a property which exclusively belongs to the action of the diphtheria bacilli. The diphtheritic process, an inflammation with the formation of a fibrinous exudation, is one of the most common of pathological processes. We find it on all of the surfaces of the body. It is more commonly seen in the action of certain bacteria than others. Among the bacteria the pneumococcus is probably more apt to produce a fibrinous exudation than any other. The most typical fibrinous exudation may be produced by chemical irritants; the application of croton oil or of the vapor of ammonia to the mucous membrane of the pharynx or trachea will produce a typical fibrinous exudation.

The writer once saw a case in which an extensive membrane, extending into the larynx and trachea, was produced by the accidental application of croton oil. A man was brought into the hospital unconscious, in a condition of uremic coma. A few drops of croton oil were applied to the back part of his tongue. The patient accidentally made an inhalation at the same time, and the croton oil was aspirated into the air-passages. After death the entire surface of the trachea and larynx extending down into the finer bronchi was covered with a dense membrane which could not be distinguished from the membrane of a marked case of diphtheria.

A great deal of interest has come from the study of the cases of diphtheria at the autopsies at the City Hospital. It is known with regard to many bacteria, while their action is exerted more particularly at certain places, this being due to external conditions favoring their entry into the organism at certain places, and to the fact that in these places they find more suitable conditions for their existence, they may be found also in other uncommon situations. This is notably the case with the pneumococcus. There are few pathological conditions found in any part of the body which may not be associated with this organism. While the diphtheria bacilli are always found associated with the typical disease having its local manifestations in the pharynx or air-passages, they are more rarely met with in other parts of the body and associated with other processes.

It is our habit to make bacterial cultures at all autopsies from all of the principal organs of the body. In one case of acute endocarditis, typical Klebs-Löffler bacilli were found in the heart valves. These bacilli, morphologically, and in all of their cultured relations, were identical with the ordinary bacilli, but were devoid of virulence. They were associated with pneumococci, and the lesions were probably due to these. A similar case has been reported by Howard. His case was also one of endocarditis. The bacilli were found in the vegetations on the cardiac valves and in all of the organs. They were identical with the Klebs-Löffler bacilli with the exception of being non-virulent. In another case they were obtained from the lungs in a case of disseminated tuberculosis. There was a tuberculous ulcer of the trachea from which no cultures were made, nor were any made from the pharynx. In the culture from the lungs numerous colonies of diphtheria bacilli and streptococci developed in addition to a few colonies of staphylococcus aureus and other organisms. Streptococci were also found in the spleen. The bacilli killed a guinea-pig in thirty-six hours with the lesions of experimental diphtheria. From another case of a lung in a state of gray hepatization, the culture made from a consolidated area of the lung gave a few colonies of the Klebs-Löffler bacilli along with other organisms. A guinea-pig inoculated died at the end of three weeks. In a fourth case, one of extensive phthisis, the autopsy made at one of the insane asylums, a few colonies of Klebs-Löffler bacilli developed from the lungs. Unfortunately in but one of these four cases were the throat organs examined. The circumstances of the autopsy forbade the examination of the throat, but there was nothing clinically which directed attention to the throat. In the one case in which the throat was examined a typical tuberculous ulcer was found, from which, however, no cultures were made.

These cases are interesting, and it is difficult to fully explain them. The general result of cultures at autopsies shows that in most diseases there is a poly-infection when death takes place. Not only are the specific organisms of the disease found, but there are other organisms whose presence is more or less accidental and which have had little to do with the production of the lesions. They are usually the more common organisms which find opportunity for entering the tissues in the reduced resistance preceding death. It is probable that these four cases should be so regarded. Other organisms were present, and the lesions were not of such a character as to lead us to believe that the diphtheria bacilli had any part in their production. These cases are to be distinguished from true mixed infections in which distinct lesions due to the action of various organisms have been found. In the cases investigated at the City Hospital such mixed infections have been seen in typhoid fever, scarlet fever and measles; the lesions in these diseases being accompanied by throat lesions due to the diphtheria bacilli.

Diphtheria bacilli may be found at other places on the surface of the body. The intact epidermis of the surface of the body probably is an efficient protection against their action, but superficial abrasions or ulcers on persons with diphtheria are apt to become infected. The bacilli have been found in an anal fistula, in an ulcer on the arm, in excoriations on the face, and in a number of other places on the surface. The mode of

infection is by the hands which may be assumed to be always infected from contact with the secretions of the mouth and nose containing the bacilli. Wright calls attention to the slight degree of virulence of the bacilli which are found in these places. In twelve cases in which the virulence was tested the bacilli from three had no effect on guinea-pigs, and in three other cases the inoculated animals lived more than a week. Wright states that a mild degree of virulence or none at all has been found in 50 per cent. of the organisms obtained from these places.

In some of the infectious diseases the organisms causing the disease are only found in connection with the primary lesions. In others there may be a general invasion of the blood and tissues with the organisms. In the early investigations on diphtheria the bacilli were only found in the primary lesions, and it was supposed that no general invasion of the body took place. More recently, Frosch and others have found the bacilli in the internal organs by the usual methods of culture. They are present in such small numbers that they are only found by using large quantities of the substance of the organs for the cultures. Wright's statistics of cultures show that they are most frequently found in the lungs, and more rarely in other organs; but they may be found in any of the internal organs. It is natural that they should be more often present in the lungs, from the close connection of these with the larynx and pharynx. The bacilli are present in these internal organs in such small numbers that they cannot be found on microscopical examination of the tissue, nor can they be shown to have any relation with the lesions in the organs. So far as we know at present the diphtheria bacilli are only capable of producing typical lesions on epithelial surfaces. It is to be expected that a few in the course of the disease may get into the blood or lymphatic circulation, and thus be deposited in the internal organs. When directly injected into the subcutaneous tissue of rabbits they induce extensive necrosis of tissue and inflammation.

We come now to the second class of lesions found in diphtheria, those produced not by the direct action of the bacilli on the tissues, but by the action of soluble chemical substances which are produced by the bacilli in the primary foci, and absorbed from there and carried to the tissues everywhere. Some of the lesions so produced are evident on macroscopic examination, others are only found on microscopic examination. Oertel, in his classical monograph on the disease, was the first to call attention to these lesions. Welch and Flexner have called attention to similar lesions in experimental diphtheria, and have shown that they may be produced by injecting into animals the soluble toxins derived from cultures of the bacilli. The most obvious of these lesions are found in the lymph glands of the body, and consist in hyperplasia of the lymphatic tissue. The lymph glands of the neck into which the lymphatics from the throat enter are the most affected, but all the lymphatic tissue, including the follicles of the spleen, take part in it. In the intestinal canal both the solitary follicles and the Peyer's patches are greatly enlarged, and the latter may present the appearance of the early stage of typhoid fever. The swollen glands are red and soft.

The spleen is somewhat enlarged, this being due to hyperplasia of the follicles and of the pulp. It is never so soft as in some of the other infectious diseases. The liver rarely shows much change to the naked eye.

It is somewhat paler than normal, and occasionally small opaque spots may be seen in it. The kidneys are rarely affected. In forty-one autopsies on diphtheria, in but one case an acute interstitial nephritis was found. Diphtheria has not the same tendency to produce nephritis as have the other infectious diseases of children. Slight degenerative changes are found on microscopic examination sufficient to explain the albuminuria commonly present. In two of our cases ecchymoses were found in the mucous membrane of the stomach, and the bacilli obtained from them on culture.

On microscopic examination lesions consisting in small areas of necrosis, with nuclear fragmentation, may be found in all the organs. They are more common and more marked in the lymph glands than elsewhere. Oertel was the first to call attention to them. From our experience it would seem that they are more common in the experimental diphtheria produced in animals by inoculation than in man. These foci are not produced by the action of the bacilli. On microscopic examination no organisms are found in connection with them, and they may be produced by the soluble toxins obtained from cultures. They are of the same general character as the small necrotic foci found in the liver in typhoid fever, and which are produced not by the bacilli but by toxic substances absorbed from the alimentary canal.

We come now to the consideration of the third class of lesions, those due not to the action of the specific bacteria and their soluble products, but to other organisms which find favorable conditions in the primary lesions produced by the diphtheria bacilli. There is little doubt that in most cases, particularly in the later stages, there is a mixed infection in the primary lesions of the disease. It is very seldom that a pure culture of the diphtheria bacilli can be obtained from the throat. The most common pathogenic organism found with it is the streptococcus pyogenes, one of the pyogenic organisms. We do not know what part this organism plays in the primary process. It is quite capable of itself of producing a diphtheritic inflammation, which cannot be distinguished from that produced by the diphtheria bacilli. It is possible that it assists in the production of the membrane, or it may in some way favor infection with the bacilli. These other organisms act in two ways. They may be taken up from the throat by the blood and lymphatic vessels, enter into the circulation and produce a general septicemia, or they may pass from the primary foci into the lungs. Wright has found in twenty-six out of forty-one autopsies a more or less general invasion of the blood and tissues with the pyogenic organisms, among which the streptococcus was the most common.

Broncho-pneumonia is one of the most common conditions met with in the infectious diseases of children. It has been present in greater or less degree in twenty-four of the forty-one autopsies. Its cause must be attributed to the action of the streptococci. It is true that the diphtheria bacilli are found in the lungs in nearly all autopsies on diphtheria, and they may produce a typical membrane in the fine bronchi. On microscopic examination of the tissue they are not found in the alveoli in connection with the lesions, and from what we know of the pathogenesis of the bacilli it is not probable that they could produce such lesions. The streptococci or pneumococci in these cases are found in cultures made from the lungs and, on micro-

scopic examination, in connection with the lesions. Moreover, similar lesions are found in scarlet fever and in measles. In many of the autopsies these pulmonary lesions are so extensive that death may be directly attributed to them. It would not be possible in the limits of this paper to consider the very interesting questions of the comparative virulence of the diphtheria bacilli and the characters pathological and clinical of those cases of angina which resemble diphtheria and are not produced by the diphtheria bacilli. Such cases are not infrequently met with. On the other hand, it should be remembered that inflammation produced by the diphtheria bacilli does not always lead to the formation of a pseudo-membrane. Cases have frequently been seen in which a simple angina has been associated with the bacilli. From the anatomical and experimental study of diphtheria we may regard as certainly established:

That there is a definite infectious contagious disease produced by the diphtheria bacilli.

Other organisms may produce lesions in the throat which are similar to the primary lesions of diphtheria.

The primary lesions of the disease are due to the action of the diphtheria bacilli. They are always found associated with these lesions, and are chiefly found on the surface or in the upper layers of the membrane.

There may be a more or less general invasion of the tissues by the bacilli. They are found in the internal organs in small numbers, and do not appear to have any relation to the lesions.

Lesions are produced in the internal organs by the action of soluble toxic substances which are produced by the bacilli in the primary lesions and absorbed. Similar lesions can be produced in animals experimentally by the injection of the germ-free filtrates of cultures.

Cultures made at the autopsies have shown that in most of the fatal cases there is a general invasion of the tissues by some of the pyogenic organisms, of which the streptococcus is the most common.

Infection with other organisms plays a considerable rôle in the pathology of the disease, and some of the most important lesions, as the broncho-pneumonia, are due to these other organisms.

There is not the same tendency to renal affections in diphtheria as in scarlet fever and other infectious diseases of children.

Abrasions of the skin in patients with diphtheria are apt to become infected with the bacilli.

THE PREPARATION OF DIPHTHERIA ANTITOXIN.¹

BY JOSEPH L. GOODALE, M.D., BOSTON.

THE general principles involved in the preparation of diphtheria antitoxin have recently become familiar to all medical men. Their recapitulation does not therefore seem essential for the purposes of the present paper, which are, first, to note the events of interest occurring during the prosecution of some recent work on the subject; and second, to direct attention to certain points of difficulty which have been encountered.

In view of the limited time at my disposal it appears

¹ Read at the Annual Meeting of the Massachusetts Medical Society June 12, 1895.

preferable to omit detailed reference to the preparation of culture media and to the selection of special bacteriological apparatus. It will be sufficient to state that full accounts of the former are to be found in numerous recent works, while complete catalogues devoted solely to this subject have been issued by several manufacturers of scientific supplies.

The acquisition of a culture of diphtheria that will yield the requisite amount of toxine under specified conditions is a matter of the first importance. One culture may be a hundred times as productive of toxine as another, although both are grown under the same conditions. My own experience indicates that a satisfactory culture is something of a rarity. Cultures were tested from eight cases of severe clinical diphtheria in the following way: From each case a flask of bouillon was inoculated, and the virulence of the fresh twenty-four-hour culture tested by injecting into a guinea-pig one cubic centimetre. From the two pigs which died first, within two days, pure cultures of the diphtheria bacillus were again recovered, inoculated into bouillon, and grown under proper conditions for three weeks. At the end of that time the bouillon of one flask was found to be nearly twice as rich in toxine as the other, and this culture was used exclusively in the subsequent work. The virulence of this bacillus and its toxic productiveness have remained unimpaired after more than a hundred re-inoculations, covering a period of eight months.

When a flask of bouillon is inoculated with the diphtheria bacillus the following phenomena occur: At the end of a day or two the bouillon is generally turbid throughout, but with little or no sediment. Although the bacilli are now at the stage of maximum virulence, yet no appreciable amount of toxine is to be found, as may be shown by filtering off the bacilli, and injecting the clear filtrate into guinea-pigs. Large doses of this produce often no more effect than an equal amount of ordinary bouillon. At the end of a week a distinct sediment settles, and a thin translucent layer of bacilli can be seen floating on the surface of the liquid. Microscopical examination shows what is taking place: The sediment consists of dead bacilli, disintegrated, and no longer identifiable, while the surface layer is composed of living, rapidly multiplying bacteria of characteristic appearance. Toxine can now be demonstrated in the fluid.

During the next few weeks the sediment increases, often forming more or less coherent flakes and lumps, the superficial layer becomes thicker and more opaque, and is composed of scaly particles. If these be once separated by shaking they show no tendency to coalesce, but remain discrete while the intervening surface becomes covered with a film, slowly and progressively increasing in opacity. After five or six weeks the surface layer often sinks and disappears; examination shows very few living bacilli, but the fluid is rich in toxine.

It has been stated that toxine is not secreted by the living bacilli, but is the product of their disintegration after death. Whether this be so or not, the amount of toxine in a given bouillon culture is proportionate to the amount of sediment, that is, to the number of bacilli which have completed their cycle in the liquid. Multiplication of the bacilli is favored by access of oxygen, and it has been consequently recommended to grow the bouillon cultures in a current of air. It is unquestionably true that a bouillon culture three