Memoir on the nature of diphtheria / by H.C. Wood and H.F. Formad.

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MEMOIR ON THE NATURE OF DIPHTHERIA.

By Drs. H. C. WOOD and H. F. FORMAD,
OF PHILADELPHIA.

APPENDIX A, REPORT OF THE NATIONAL BOARD OF HEALTH FOR 1882.

With Compliments of
H. C. Wood

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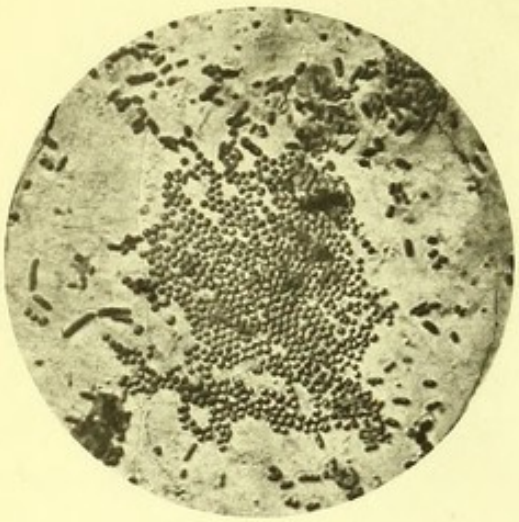

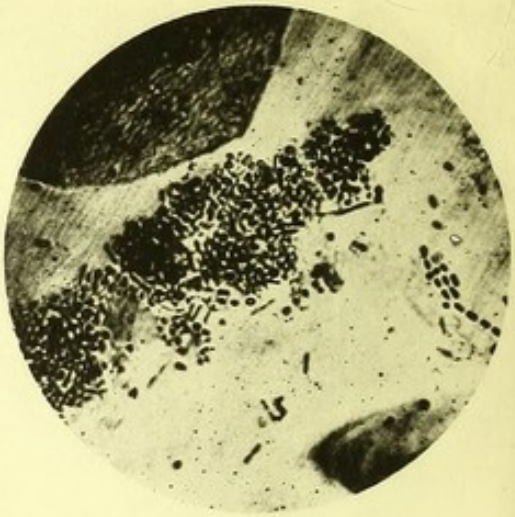

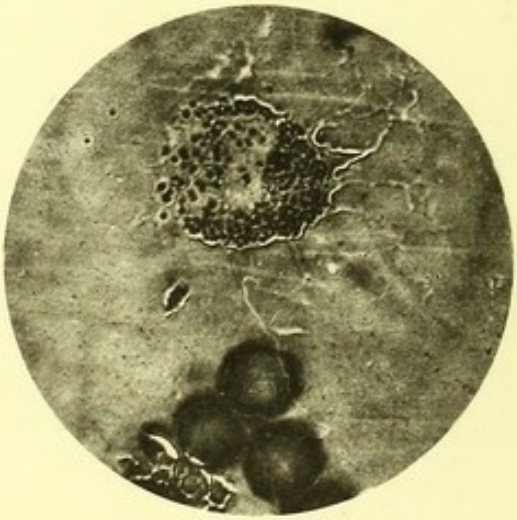

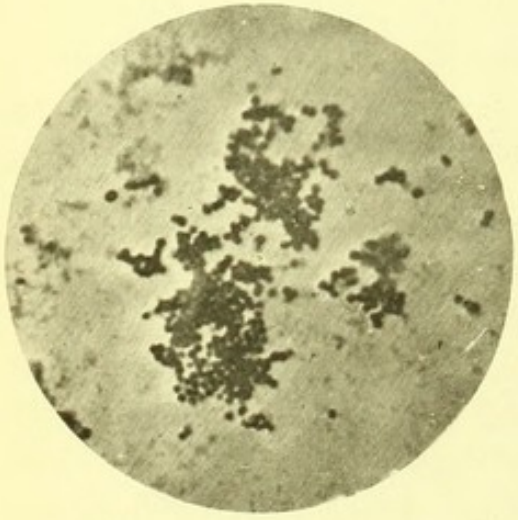

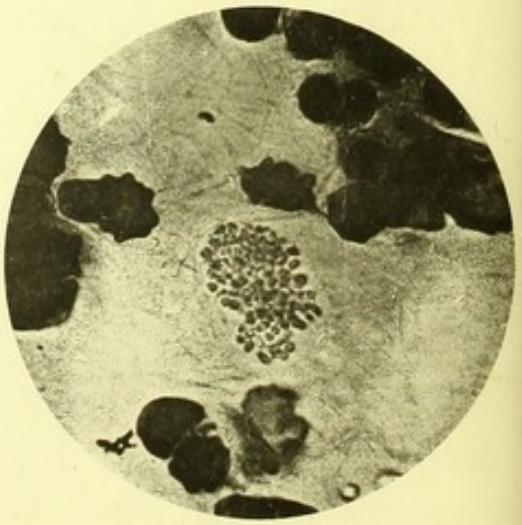

PREFACE.

THIS memoir was sent to the National Board of Health, in the form now published, about September 20, 1882, and of course contains no reference to articles of later date. Since that time Professor Klebs has claimed (*Verhandlungen des Congresses für innere Medicin*, Wiesbaden, II. Abth., 1883) that there is a bacillus in the false membranes of diphtheria, and Dr. Löffler (*Mittheilungen aus dem Kaiserlichen Gesundheitsamte*, Bd. II., 1884) has also asserted the presence of such an organism. Dr. Löffler states, however, that bacilli were absent in a number of typical cases of diphtheria, and offers no conclusive evidence that they have etiological relations with the disease. Further, it does not appear that Dr. Löffler ever found the bacillus in the freshly-poured-out membrane, and we think that his bacilli are the same as our rod-bacteria, which, for reasons sufficiently stated in the memoir, we believe to be connected with putrefactive changes in the exudate, and not to be the virus of the disease. The prize essay of Dr. Huebner, entitled "*Die Experimentelle Diphtherie*" (Leipsic, 1883), does not seem to us to require discussion here.

A very important fact, which has only become known to us since the completion of our memoir, is the apparent passage of diphtheria back from our infected animals to a human being. A young son of Dr. Michael O'Hara visited the laboratory and participated in one or two post-mortem examinations upon infected rabbits. Four days after this he was taken with diphtheria, which ran a moderately severe course. There was no other known exposure in this case, and the father of the child is very fixed in the belief that the disease was contracted from the rabbits.

In the text of this memoir are wood-cuts purporting to be reproductions of the micro-photographs made in the University Laboratory by Dr. William Gray. The cuts are so imperfect that we have added, as a frontispiece, an autotype of some of the original photographs. The upper figures correspond to Figs. 5 and 6 of the text; the central, to Fig. 7; the right-hand lower figure, to Fig. 8. They represent—Fig. 5, micrococci from blood in diphtheria; Fig. 6, micrococci from normal mouth, mixed with rod-bacteria; Fig. 7, a zoogloea mass from a freshly-burst corpuscle; Fig. 8, an earlier stage of the same, the corpuscle not burst; the remaining figure, micrococci from cultivation—all magnified 1000 diameters.

UNIVERSITY OF PENNSYLVANIA, June 9, 1884.

APPENDIX A.

MEMOIR ON THE NATURE OF DIPHTHERIA.

By Drs. H. C. Wood and H. F. Formad, of Philadelphia, Pa.

PREFACE.

The research of which the present memoir is the outcome was originally undertaken at the instance of the National Board of Health, and has been prosecuted under their auspices. In detailing and discussing the results we have divided the subject into several chapters, more or less arbitrarily it may be thought, but yet, as we trust, in manner to add to the completeness and perspicacity of the memoir.

University of Pennsylvania, *October* 1, 1882.

CHAPTER I.

ON THE STRUCTURE OF THE DIPHTHERITIC MEMBRANE.

Experimental studies made upon the lower animals and careful histological studies of the morbid process as it occurs in the human subject have convinced us that a

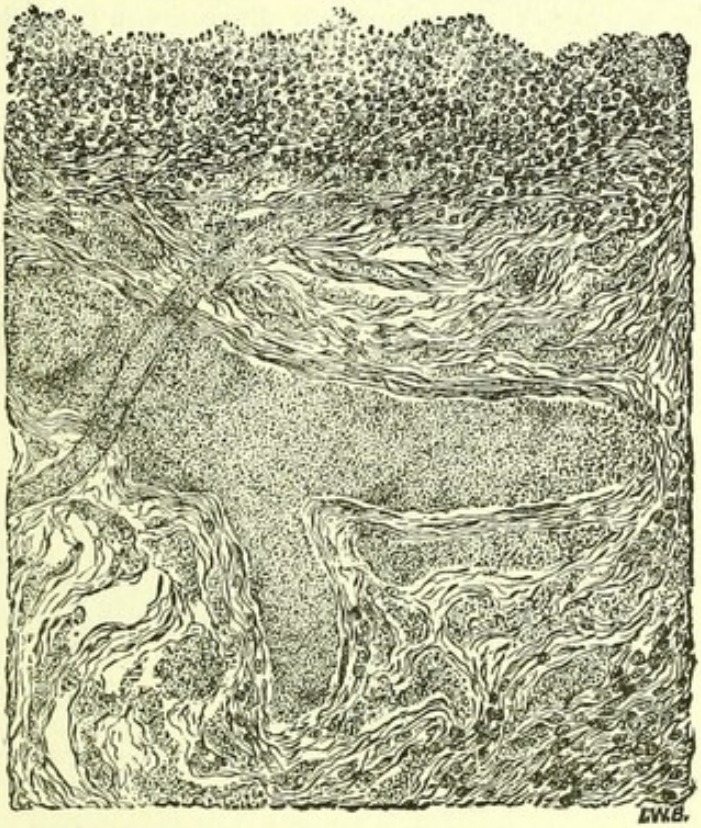

Fig. 1.—Transverse section through the floor of a diphtheritic ulcer of pharynx of child, representing submucous tissue with blood vessels and lymph-spaces filled with micrococci. Part of the necrotic mucosa is seen in upper portion, the bulk of it having been removed together with the pseudo-membrane. Magnified 250.

pseudo-membrane has a similar constitution in whatever part of the body it may have been formed, and whatever may have been the cause of the inflammation which gave rise to it. The slight differences which exist are due to the various structure of the parts affected, to the degree of the inflammatory process, and to the opportunity which has been afforded for the development of microscopic organisms. In the present chapter we will therefore not employ the terms diphtheritic and croupous membranes, but pseudo-membranous exudates, which, it is to be rembered, may be situated superficially upon a membrane or part, or may be deep seated in a tissue. Many clinicians consider

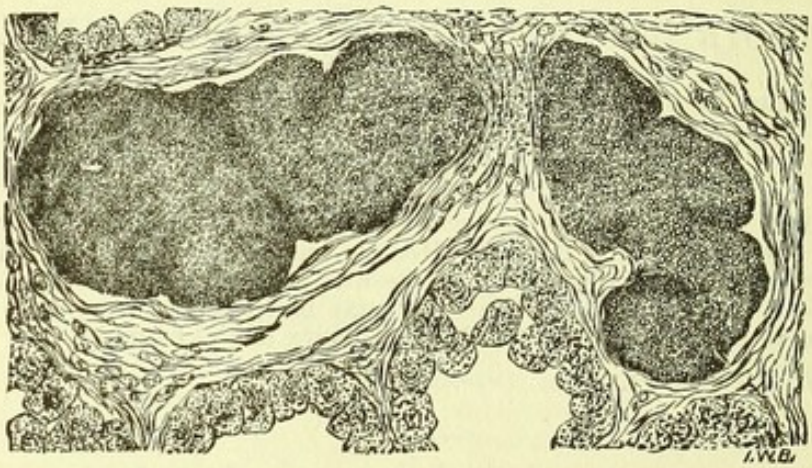

FIG. 2.—A micrococcus-embolus obliterating a small vein in kidney, from case of diphtheria in child See case 5. Magnified 500.

diphtheria and pseudo-membranous croup to be distinct diseases. The discussion of this question must be postponed until the latter part of this memoir, : at present we are only concerned with the anatomical point of view, i. e. with the structural characters, and with the relations of the exudates.

It is easy to demonstrate that the apparent difference in the lesions of diphtheria and pseudo-membranous croup, and in the morphology of the exudates, is partly dependent upon the anatomical peculiarities of the pharynx and respiratory passages, and partly upon the degree of the inflamation.

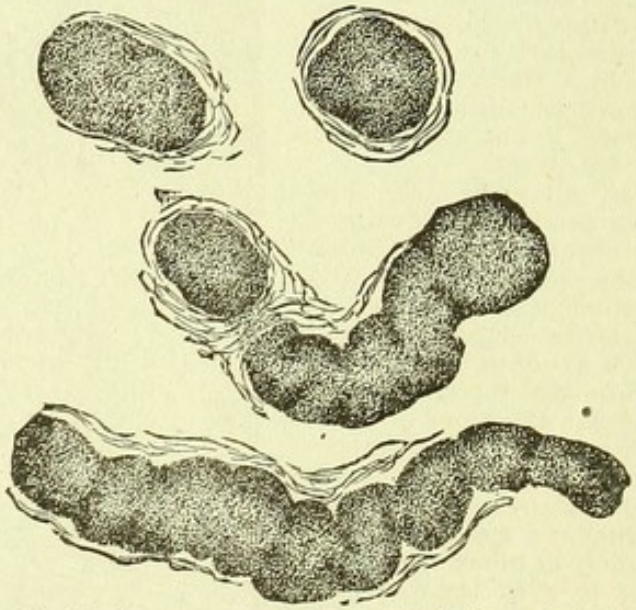

FIG. 3.—Transverse and longitudinal view of micrococcus-emboli from kidney. See case 22. Magnified 300.

The submucous tissue of the pharynx is made up of a loose, highly vascular connective tissue, which does not restrict the congestion of the vessels and the extravasation of the corpuscular elements. At the same time the rapidly coagulable exudate only partly reaches the surface of the mucous membrane, the bulk of it being kept below by the heavy layer of stratified epithelium.

The submucous tissue of the trachea, on the other hand, is made up of a dense elastic tissue and of an only slightly vascular cellular tissue, backed by the avascular

cartilaginous rings. Hyperæmia and extravasations from the vessel are here limited, and only severe inflammation will give rise to exudates, which, when produced are rapidly expelled by the dense matrix, and, meeting with no resistance on the part of the single-layered epithelial covering, coagulate mostly on the surface.

Again, the mucous membrane of the fauces and mouth has a squamous not easily detached epithelium, and consequently membrane connected with or springing from such surface is firmly adherent. The epithelium of the trachea is columnar, ciliated, and detaches with the utmost facility even in normal conditions of the organ; hence exudate attached to it separates readily. The exudate of diphtheritic trachitis is always readily detached in the line of the epithelium. The detachment is, according to Rindfleisch, further facilitated by the excessive secretion of the tracheal glands, the liquid forcing itself between the mucous surface and the pseudo-membranous exudate.

Another distinguishing point made by authors between the pharyngeal and tracheal lesions in question is the independence of their individual occurrence, it being asserted that, if a real malignant diphtheritic trachitis occurs, it is only by extension from the pharynx downwards into the air passages. It is true that diphtheria usually begins in the fauces, but we have observed in several instances the reverse to have taken place; and in two of our rabbits in which an artificial pseudo-membranous trachitis was induced the disease extended also upwards, producing a secondary most violent pseudo-membranous angina.

As an important difference between diphtheritic and inflammatory croup, and between diphtheria and the so-called true croup, some authorities insist upon the presence of micrococci in one and their absence in the other affection.

This is, however, an error. We have never been able to produce artificially in the animal or to obtain from the human subject a membrane free from micrococci, although under some circumstances the micrococci are very much fewer than in ordinary diptheritic exudate.

The frequent absence of the systemic disturbances in the local affections of the air passages can likewise also be explained by the anatomical peculiarities of the trachea and larynx; the dense unyielding subcutaneous and cartilageous tissues of these parts, their deficiency in blood vessels, and imperfect connection with the lymphatic apparatus, prevent in a great measure the absorption into the system of the necrotic products, so that either recovery or death from stenosis usually takes place before a systemic infection ensues. In some instances in which the tracheal affection has lasted long enough, we have seen distinct systemic infection manifested by the symptoms and evident from the presence of micrococci in the blood.

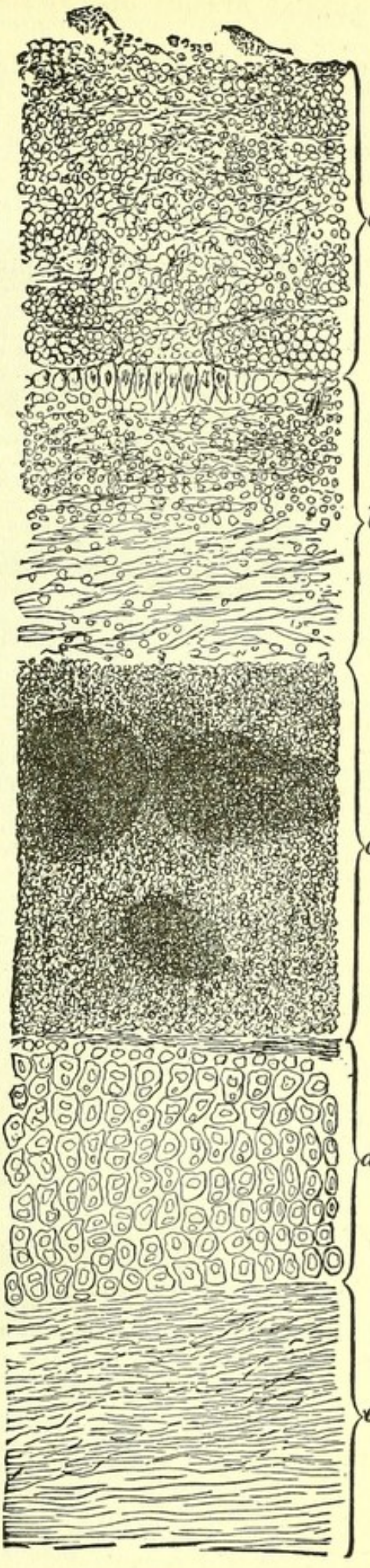

FIG. 4.—Transverse section of trachea with artificially-produced (by ammonia) pseudo-membrane. Magnified 250 diameters. *a*. Superficial pseudo-membrane. *b*. Croupous exudate below basement membrane. *c*. Ecchymosis in submucous-tissue and infiltration of tracheal glands with blood corpuscles. Between *a* and *b* are seen some remains of surface epithelium. *d*. Cartilage. *e*. Outer connecting tissue and muscular investment.

A subdivision of membranous croup into a diphtheritic and inflammatory variety is anatomically not justifiable.

We have observed that the exudation on the trachea, even in the simplest inflammatory croup, is met with not only on top of the epithelium, but also below the latter, precisely as in true diphtheritic angina, only in a slighter degree.

Our preparations show that the exudation of the croupous inflammation excited artificially in the trachea is not merely superficial, but also extends below the basement membrane, as illustrated in Fig. 4.

In cases of artificial croup as well as in those observed in man, in which the *submucous* infiltration is prominent, we have seen loss of substance, viz, necrotic ulcerations similar to those occurring in genuine diphtheritic angina.

It would seem therefore that there are no specific anatomical characters in the diphtheritic exudate which separate it from other acute false membranes; also that micrococci are always abundant in the exudate, usually forming a great part of its bulk.

The microscopic picture obtained from our sections, made perpendicularly through diphtheritic ulcers, show that simple inflammatory changes are the initial step in the local diphtheritic process. This is also in accordance with the description of all reliable observers.

All the peripheral layers of the part affected are seen to be intensely infiltrated by the pseudo-membranous exudate, all the blood vessels are obliterated, and life and nutrition of the part nearly ceases. The tissue undergoes fatty degeneration, and hence is quite easily penetrated by the micrococci on the surface. This produces a condition histologically not unlike gangrene and probably identical with it. The micrococci advance from the periphery inwardly to enter the lymph-spaces opened through destruction of the connective tissue. Once having entered the lymph-spaces, the micrococci are not seen to produce any histological changes in the surrounding deeper tissues. They next enter the blood vessels. While all the lymph-spaces are overdistended with micrococci, the blood vessels can be frequently observed to be empty side by side with the lymph-spaces. In other places a gradual filling of the blood vessels (veins chiefly) with micrococci is seen. This evidently proves that the micrococci enter the blood through the medium of the lymph-spaces.

The drawings which we append show the way in which the micrococci enter the system. We have endeavored to make photographic illustrations, but found it impossible to bring out the structure satisfactorily. The cuts are photographic reproductions of very accurate and careful camera-lucida drawings.

Fig. 1 represents a perpendicular section through the floor of a diphtheritic ulcer of pharynx of child (age three years) who died on the seventh day of the disease (see Case No. 5 in tables). The false membrane proper had nearly altogether sloughed away and the rest of the exudate become detached during the preparation of the section, leaving the bare submucous tissue. A vein running parallel to the surface is seen completely blocked up by micrococci. The latter crowd also all the lymph-spaces of the connective tissues everywhere. A small capillary blood-vessel crossing the vein at nearly right angles is also seen to be filled by micrococci. The upper portion of the drawing represents gradually advancing necrotic change of the peripheral granulation tissue.

Figs. 2 and 3 represent camera-lucida drawing of micrococci emboli from kidneys. These emboli will be often referred to in the tables and text of this monograph.

For the sake of comparison we print here Fig. 4, which shows a narrow strip of a transverse section of trachea with artificially-produced (by ammonia) pseudo-membrane in rabbit. The exudate is seen both above and below the epithelium and basement membrane.

The results which have been arrived at in this chapter may be summed up in conclusion, briefly as follows: First. No anatomical essential differences exist between diphtheria and croup; the apparent differences being conditioned by the location of the exudate. Second. Micrococci are always present in the local diphtheritic exudate. Third. If the local diseased process goes far enough, the micrococci force their way through the lymph-spaces, opened by the necrosis, into the veins and finally into the blood. The obvious conclusion is that whilst the clinico-pathological evidence does not prove that the micrococci are essential to diphtheria, it leads towards such a view, and at least shows that some close connection exists between these organisms and the disease.

CHAPTER II.

ON THE CLINICAL RELATIONS OF MICROCOCCI TO DIPHTHERIA.

Having found that the micrococci are an essential part of the diphtheritic membrane, the important question presents itself as to whether the micrococci found in the diphtheritic lesions are identical in form and size with those which are present not only in the membrane of a non-diphtheritic trachitis, but also in the exudation of an inflamed tonsil? We do not hesitate in affirming that it is not possible to determine, even with the highest powers of the microscope, whether an individual micrococcus colony or mass of such colonies have come from the surface of a furred tongue, or from an ordinary case of simple throat inflammation, or from one of a diphtheritic character. As evidence of this we offer the following microphotographs, Fig. 5 taken from an undoubted case of diphtheria, Fig. 6 from an ordinary tongue scraping.

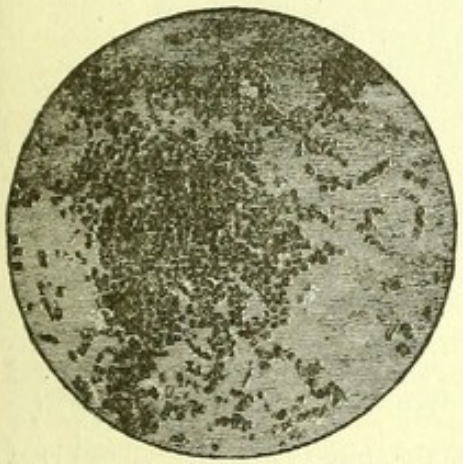

FIG. 5.—Micrococci from blood in diphtheria × 1,000.

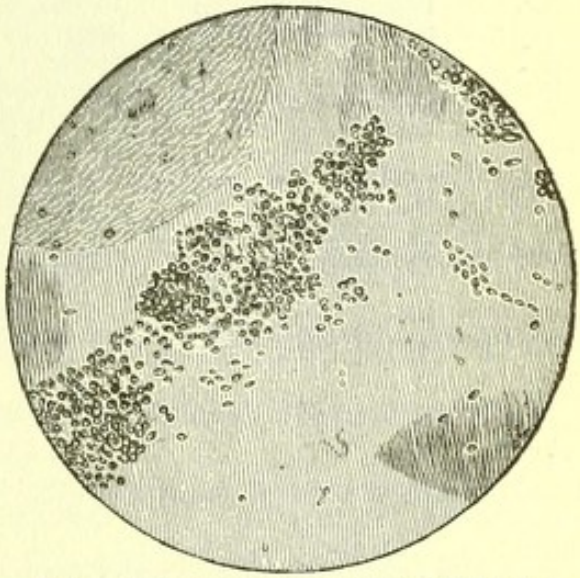

FIG. 6.—Micrococci from normal mouth × 1,000.

A second question, no less important than that just discussed, is, Are the micrococci present always in the blood of persons having diphtheria, or only in some cases? The vital nature of this query scarcely requires pointing out. If micrococci are really always to be found in the disease it is very probable that they are at least an essential part of it; on the other hand, if they are only occasionally present it is probable that they are an accident of the affection.

In attempting to answer the question just propounded we have examined a large number of cases. Without further discussion, we desire once for all to state that every precaution has been taken to make the examinations at once careful and exhaustive.

The cases examined are naturally divided into two sets, according to their geographical and epidemic relations. One of these sets (No. 1) represents what we may be allowed to call endemic or sporadic diphtheria. The other series (No. 2) illustrates true malignant epidemic diphtheria. In the city of Philadelphia there are always more or less numerous cases of the disease of varying type as to severity, but at no time during our studies has there been anything which could properly be termed an epidemic of diphtheria. Cases of set No. 1 were all observed in Philadelphia or its vicinity.

The town of Ludington, situated on Pere Marquette Lake, upon the east shore of Lake Michigan, in the State of Michigan, is a center for the lumber trade, and an enormous amount of sawdust is annually produced there. A part of the town is situated upon a high plateau, but the third ward, bordering upon the lake, is built upon a swamp, which has been filled largely with the great staple of the place—*i. e.*, sawdust. The drainage is so bad that in many places a hole dug a couple of feet in the ground soon fills with water, and only in a small percentage of the houses has any attempt been made to construct cellars. During the winter of 1880 and 1881 diphtheria appeared in this low region and spread with such thoroughness that it is said scarcely a child escaped an attack, and about one-third of them died. Dr. Formad was sent in the month of June to Ludington, and made studies there of the disease, with espe-

cial reference to the micrococcus question. In the spring of 1882, Dr. Formad went to Lakeview, in the center of Michigan, to study an epidemic at that place.

The blood examinations during life were made directly at the bedside. They were all purely microscopical, and attention was especially directed to the presence or absence of micrococci, and, when they were found, to their mode of grouping, &c.

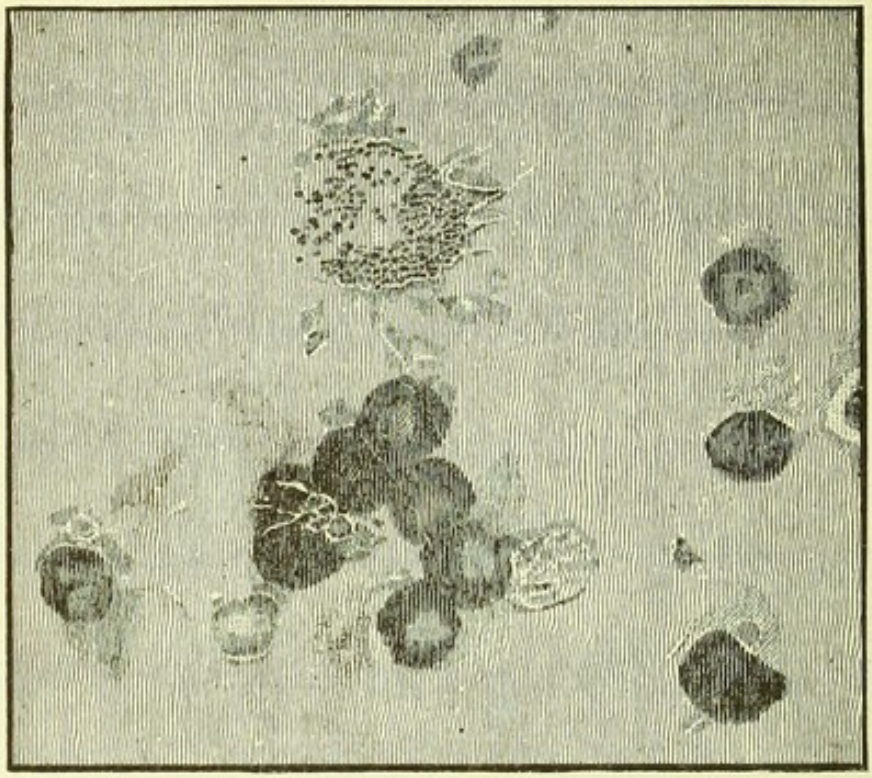

FIG. 7.—Micrococci in blood from diphtheria × 1,000, showing a zooglæa mass.

In all cases the blood was taken from the finger with due precaution as to cleanliness. Uniformity of method was strictly observed in the examinations. In doubtful cases caustic potash, glacial acetic acid and aniline dyes were used as tests for micrococci.

As a rule in all fatal and in many favorable cases the quantity of the white blood corpuscles was found increased; the average proportion of white to red was frequently

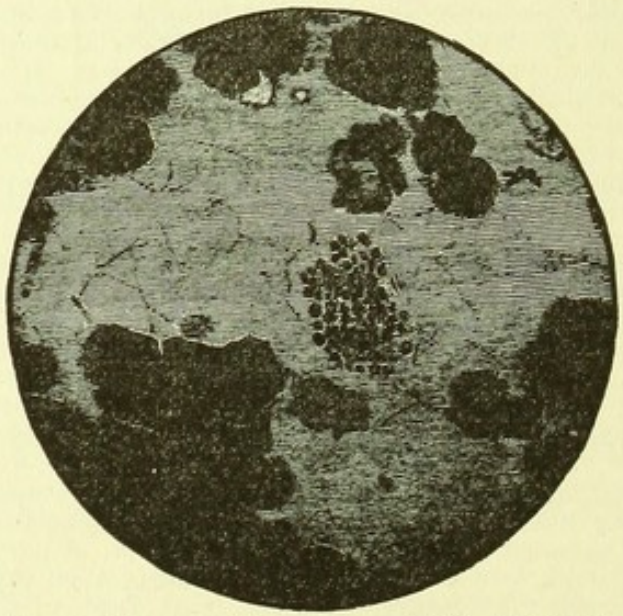

FIG. 8.—Micrococci in blood from diphtheria × 1,000, showing a zooglæa mass.

about one to fifty and occasionally one to twenty. Great irregularity in size and coarser granulation than normal of the protoplasm of the corpuscles was observed in all bad

cases and only rarely in favorable ones. A peculiar appearance of the white blood corpuscle was also observed, which we will call "loculated." Such white corpuscles contained vacuoles usually intercepted by delicate partitions, and were usually irregular, lobulated, enlarged, and as a rule contained a few to many micrococci.

Nearly all cases of diphtheria in which these modified white blood corpuscles were observed terminated fatally, and all such cases showed large quantities of micrococci in the blood. Sometimes these corpuscles appeared, as it were, ruptured and zoogloea-masses of micrococci were seen alongside or in their immediate vicinity. The red blood corpuscles very seldom form rouleaux in diphtheritic blood, appearing also paler and apparently losing their bi-concavity. They are never affected by micrococci. Precipitation of fibrin is unusually well marked in the blood from diphtheritic cases.

For the opportunity of examining the cases, particular thanks must be tendered to Drs. Lamareau and Adams, of Lakeview, Mich., and to several physicians of Philadelphia, especially to Dr. Jno. M. Keating, of the Philadelphia hospital.

The reproductions of the microphotographs in this paper are not successful, and do not fairly represent the originals sent with the report. The original microphotographs were executed in our laboratory by Dr. William Gray.

No. 1.—*Cases of diphtheria studied in Philadelphia.*

No. of case.	Sex.	Age.	Day of disease.	Condition of blood, quantity and character of micrococci.	Quality and condition of white blood corpuscles.	Condition of patient at the time of examination and termination of disease.	Remarks.
1	Male....	3 years..	.....	Blood did not show micrococci during life.	.....	Died on fifth day..	*Autopsy.*—Blood taken by sealed glass tubes from jugular vein eighteen hours after death contained micrococci; organs not examined; false membrane from larynx used for culture. Last examination of blood was two hours before death. Died from laryngeal stenosis.
2	....do ...	6 years..	Fifth day.....	No micrococci found during life; blood examined on sixth and eighth day, with the same result.	Normal	Condition of patient bad, but chiefly from local symptoms. Died on eighth day.	*Autopsy, thirty hours after death.*—Blood taken from jugular by means of sealed tubes contained micrococci both in masses and attacking the white blood cells. Rigor mortis not marked; no signs of decomposition. Face and neck were of dark-bluish hue. All the veins of the neck were enormously distended by very dark blood. Salivary and cervical lymphatic glands strongly congested and somewhat enlarged. Larynx and trachea were almost completely obstructed by casts of false membrane, which extended into many of the bronchi. The mucous membrane of the upper anterior portion of the trachea showed several shallow necrotic ulcerations. The vocal cords were thickly covered with closely-adherent pseudo-membrane. Lungs congested and œdematous; several hemorrhagic infarctions at the periphery of lower lobes. Pericardium and pleura congested. Heart: Right side filled with large ante-mortem clots, extending into pulmonary artery and its ramifications; orifices and endocardium normal. Liver, kidneys, and spleen strongly congested; microscopic examination did not show any other lesion than cloudy swelling, and proved absence of micrococci. Stomach empty, and, like the intestines, was much distended by gases; the mucous membrane of both congested. Other abdominal viscera normal. Examination of tongue, pharynx, and brain was not permitted.

3	Male....	11 mos ..	Third day	The blood showed abundant micrococci in large loculated vesicles, about twice the size of white blood corpuscles, out of which they were apparently formed.	White blood corpuscles increased in number.	Child very ill	Admitted to the Philadelphia Hospital July 7, with considerable membrane in the pharynx, and marked febrile disturbance.
			Fourth day	Blood as on the third day...	.....	.....	
			Eighth day	Very few micrococci	Normal	Child better.	
			Tenth day.....	No micrococci	.....do	Convalescent.	
4	Male ...	9 mos ...	Seventh day.....	.....do	.....do	Died eighth day ..	Died from laryngeal stenosis; no autopsy allowed.
5	Female .	3½ years.	Fifth day.....	Micrococci in great quantity, both free and infesting white blood corpuscles.	White blood corpuscles greatly increased in number.	Bad.....	Pseudo-membrane covering pharynx and extending into nasal passage. There was no other case of diphtheria anywhere in that neighborhood.
			Seventh day.....	Micrococci in enormous quantity, free and in zooglæa masses.	White blood corpuscles very numerous, infested by micrococci and loculated.	Worse.	
			Eighth day	Blood highly coagulable....	.....	Died in evening of the eighth day.	Autopsy made in this case ten hours after death. Blood from heart showing apparently a smaller quantity of micrococci than examination from tip of finger during life; also fewer white blood corpuscles, though all loculated. Veins of neck enormously overfilled with blood; lymphatic glands and tonsils greatly enlarged. Heart congested. Large ante-mortem clot in right side of heart, with thrombi extending into pulmonary vessels. Base of both lungs congested. Abdominal organs all congested. Kidneys enlarged and lobulated; the latter condition was probably congenital, as no structural changes could be discovered, save a slight catarrhal condition. Micrococci thrombi, obstructing many of the capillaries and small veins of kidneys; also numerous micrococc masses were seen, in smaller and larger vessels of liver, spleen, thyroid, lymphatic glands, and heart muscles. In all these last-named organs the micrococci were not as distinct thrombi. Inoculations of animals with membrane from this case nearly all proved fatal.
6	Male....	3 years..	First day	No micrococci	Blood normal	Bad sore throat; no fever.	The child was born and nursed in the colored obstetric ward; no other case was known to have occurred within month.
			Second day	.....do	Some increase of white blood corpuscles; blood otherwise normal.	Worse.	
			Third day	Micrococci in blood free and in zooglaed masses in enormous quantity; also affecting white blood corpuscles.	White blood corpuscles in great excess, and many loculated.	Very bad; croupous symptoms; worse.	Heavy pseudo-membrane extending into nasal passages.
			Fourth day.....	Same as yesterday	The same	.....	

No. 1.—*Cases of diphtheria studied in Philadelphia*—Continued.

No. of case.	Sex.	Age.	Day of disease.	Condition of blood, quantity and character of micrococci.	Quality and conditions of white blood corpuscles.	Condition of patient at the time of examination and termination of disease.	Remarks.
			Sixth day			Died early in the morning.	*Autopsy, soon after death.*—Micrococci abundant in blood from jugular vein, although less so than during life. There were also some rod bacteria. Neck greatly swollen from venous congestion and enlargement of all glands. Large ante-mortem clots in heart, containing quantities of micrococci. Internal organs all congested, but the most exhaustive examination did not reveal any micrococci.
7	Female	11 years.	Third day	No micrococci	Blood normal	Favorable	Throat not very bad.
			Fifth day	do	Some increase of white blood corpuscles.	do	Reported as recovered.
8	do	5 years.	Second day	do	Blood normal	Bad case	Very extensive pseudo-membrane.
			Fourth day	Micrococci in blood free, and also in white blood corpuscles.	White blood corpuscles loculated.	Worse	
			Eighth day			Died	There was no more chance for further examination of blood. No autopsy.
9	Male	7 years.	Fourth day	No micrococci	Blood normal, but highly coagulable.	Favorable	
10	Female	2 years.	Second day	do	Blood normal	do	
			Fifth day	Micrococci in blood, but not affecting the white blood corpuscles.	Decided increase in white blood.	Condition worse	
			Seventh day	Same as before		Apparently improving.	Patient got well.
11	do	3 years.	Fourth day	No micrococci	White blood corpuscles increased in quantity.	Quite a bad case	Two children had died before in the same family of diphtheria within two weeks.
			Sixth day			Utimately recovered.	
12	Male	5 years.	First day	do	Blood normal	Tonsils covered, otherwise well.	
			Second day	do	do	Worse	
			Third day	Some micrococci free, but not in white blood corpuscles.	White blood corpuscles increased in number.	Conditions favorable.	
			Fourth day	Abundant quantity of micrococci.	White blood corpuscles increased, also some few loculated.	Conditions favorable.	No false membrane could be obtained from this case; there was very little of it at any time.
			Fifth day	Micrococci less abundant		Improving	
			Seventh day	Very few micrococci now in the white blood corpuscles.	Blood normal	do	

			Tenth day				Reported well.
13	..do	1½ years.	Second day	Some few micrococci in blood, none in white blood corpuscles.	Excess of white blood corpuscles.	Tonsils covered, otherwise well.	False membrane disappeared under treatment.
			Fourth day	None	do	Conditions good	Recovered.
14	Female .	3 years..	Third day	No micrococci	White blood corpuscles increased.	Bad	Disease had commenced with croup false membrane extended into pharynx and to tonsils.
			Fifth day	Some few micrococci in white blood corpuscles.	do	Very bad	
			Sixth day			Died mainly of croup.	No autopsy.
15	Male....	..do		Blood examined three times, no micrococci.	Normal	Recovered	
16	...do	2 years..		Blood examined twice, no micrococci.	do	do	
17	Female .	10 mos ..		Blood examined once, no micrococci.	do	do	
18	...do	1½ years.		Blood examined four times, no micrococci.	do	do	Local affection of throat very slight in all these cases.
19	...do	1 year...		Blood examined twice, no micrococci.	do	do	
20	Male....	2 years..		Blood examined four times, no micrococci.	do	do	
21	...do	2 years..		Blood examined twice, no micrococci.	do	do	

No. 2.—*Cases of epidemic diphtheria studied in Ludington, Mich.*

No. of case.	Sex.	Age.	Day of disease.	Condition of blood, quantity and character of micrococci.	Quantity and conditions of white blood corpuscles.	Condition of patient at time of examination and termination of disease.	Remarks.
22	Male....	5 years..	Second day	Moderate quantity of micrococci, a few in white blood corpuscles.	.....	Bad.....	Half an hour before death blood was taken from the finger (which was very cold); it was dark, coagulated almost instantly, and offered but few micrococci. It was then taken from the thigh, where the temperature was still high, and was found to be gorged with micrococci.
			Third day.....	Micrococci more abundant, many in white blood corpuscles.	.....	Worse.	
			Fourth day.... ...	Masses of micrococci everywhere in blood, almost all white blood corpuscles affected.	.....	Do.	
			Fifth day.....	Very dark, and full of micrococci, none observed in white blood corpuscles.	.....	Died.	*Autopsy twelve hours after death.*—Body of livid appearance; no rigor mortis; no fetid odor; no signs of decomposition. Brain: Sinuses filled with very dark blood containing enormous quantity of micrococci. (It was taken by sealed glass tubes before opening of vessel.) All the blood vessels of the brain and its membranes congested; other changes were not notable by naked eye nor by microscope. There was some increase of fluid in the lateral ventricles. The medulla oblongata and a small portion of the cord which was removed through the occipital foramen did not show any morbid changes. Neck: Lymphatic and salivary glands enlarged and highly congested. All the large veins highly distended. Blood similar to that found in sinuses of brain. Tongue removed from below, intact with the tonsils, pharynx, larynx, and trachea. The mucous membrane of all these parts was covered with moderate sized patches of pseudo-membrane, which, in some places, appeared raised over the surface, in others, showed shallow necrotic ulcerations. The same was observed upon the vocal cords and the nasal mucous membrane. In the trachea the exudation was slight, most marked at the bifurcation, and of darker color than in other parts. Lungs: Congested, with slight hemorrhagic infarctions in lower lobes; a few of the bronchi contained casts of the exudate Heart: Pericardial fluid increased; heart muscle congested; otherwise normal; right side

							contained large, white, firm clots extending into pulmonary vessels; in left side only red clotted blood. Mycotic erosions were seen upon endocardium below the pulmonary and the aortic valves. Mediastinal and bronchial glands were enlarged and congested. Liver enlarged and extremely congested; gall bladder very much distended with very dark bile. Spleen normal size, rather hard in consistence, and showing congestion and hemorrhages. Stomach showed a few hemorrhagic erosions, and contained only a few drachms of a dark semi-liquid matter. The intestines, although of dark hue, appeared normal. Kidneys were enlarged, strongly congested, and presented the appearance of acute parenchymatous nephritis. This was confirmed by microscopic examination, which revealed also micrococci emboli in the blood vessels of these organs. The bone marrow, taken from a rib, as well as the parenchyma of the spleen, was infested by micrococci. Rest of organs normal. The urinary bladder was found empty.
23	Female..	2 years..	Fifth to tenth day.	Micrococci in blood varying from day to day, as patient was better or worse.	.....	Died.....	Died on tenth day. No autopsy.
24	Male....	7 years..	.....	.....	.....	Recovered.....	Blood examined three days and micrococci found.
25	Female..	3 years..	Fifth day.....	Moderately abundant micrococci.	.....	.....	Child convalescent at time of examination.
26	Female..	4 years..	Eighth day.....	None.....	.....	.....	
27	Male....	5 years..	Fifth day.....	Abundant micrococci.....	}		
			Sixth day.....	.....do.....			
28	.....	3 years..	.....	No micrococci in blood.....		.....	.....
29	.....	6 years..	.....	.....do.....			
30	.....	9 years..	.....	.....do.....			
31	.....	12 years..	.....	.....do.....			
32	Female..	4 years..	.....	Moderate quantity of micrococci.	.....	.....	At time of examination recovering from severe attack and still much affected.

{All had the mild form of disease; the blood of each was examined once and no micrococci found.}

No. 2.—*Cases of epidemic diphtheria studied in Lakeview, Mich.*—Continued.

No. of case.	Sex.	Age.	Day of disease.	Condition of blood, quantity and character of micrococci.	Quantity and condition of white blood corpuscles.	Condition of patient at time of examination and termination of disease.	Remarks.
33	Female.	11 years.	Fourth day	Micrococci in blood free and in small clouds; very few or no white blood corpuscles affected.	No loculated cells	Bad.	
			Sixth day	Very few micrococci		Improving	The patient was not seen again, but we learned of her complete recovery.
34	Female.	10 years.	Fifth day	No micrococci	Normal, only some increase in white blood corpuscles.	Pretty bad.	
			Eighth day	do	Normal	Rapidly improving.	Throat got well and clear, only tonsils swollen. We know the patient to have made good recovery.
35	Male	8 years.	Seventh day	do	do	Recovered.	
36	Male	2 years.	Fifth day	do	do	do	
37	Female.	6 years.	Sixth day	Some micrococci in small clouds, but none affecting white blood corpuscles.	Decided increase in white blood corpuscles.	do	Both children in one family. They were quite ill, but recovered. One child had died a few days before in the same family.
38	Male	5 years.	Third day	No micrococci		do	
39	Male	5 years.	Fourth day	Micrococci in blood and loculated cells containing micrococci.	White blood corpuscles in great excess.	Very bad; pronounced hopeless at the time.	Information concerning the exact termination of the case not obtained.
40	Male	4½ years.	Fifth day	No micrococci	Normal	Favorable	Blood was sent to us on slide by the attending physician.
41	Female.	5 years.	Second day	do	do	do	Same as last.
				do	do	do	
42	Female.	8 years.	Third day	Large quantity of micrococci in blood.	White blood corpuscles in excess.	Very bad	Termination unknown.
43	Female.	4 years.	Fourth day	Micrococci in blood; no loculated cells.	do	Very bad case	Do.
44	Female.	12 years.					
45	Male	2 years.	First day	No micrococci	Normal	High fever; bad case.	Do.
46	Male	4½ years.	Second day	do	do	Apparently bad case.	Do.
47	Female.	3 years.	Second day	do	Normal. Some slight excess of white blood corpuscles.	Bad case	Do.
48	Female.	20 years.	Third day	Micrococci in large quantity, infesting white blood corpuscles; also free micrococci, and in large isolated clouds or zoogloea masses of unusual size.	Great increase of white blood corpuscles; many loculated.	Bad	

			Fourth day	The same as yesterday	More loculated corpuscles	Somewhat better	
			Fifth day	Micrococci increasing rapidly in quantity.	White blood corpuscles increasing in quantity, but about one-third of them are loculated, and contain micrococci.	Worse	In this case there was enormous, rapid development of pseudo-membrane in pharynx, on tongue and on buccal mucous membrane, and extending into nasal passage vigorously, and to some degree into larynx. Temperature at night reaching 103° Fahr.
			Seventh day	Apparently decided diminution in micrococci.	Fewer white blood corpuscles.	Better	
			Eighth day	Same as last	....do	....do	
			Ninth day	Micrococci again, in increased quantity, in all forms of grouping.	White blood corpuscles severely attacked by micrococci.	Worse	
			Tenth day	Micrococci seen in large clouds; many also in chains and free.	White blood corpuscles in large quantity, but nearly all loculated and infested with micrococci.	....do	In the same family there had been several cases of diphtheria before.
			Thirteenth day			Died	No autopsy.
49	Female.	16 years.	Third day	Blood appears normal	White blood corpuscles infested by micrococci; no loculated white blood corpuscles.	Doing well	Moderate amount of pseudo-membrane upon ulcerated tonsils.
			Fifth day	Large amount of micrococci in blood, but only few in cells.	Great increase in white blood corpuscles; only few loculated.	Very bad	One ounce of pseudo-membrane was removed from pharynx, nose, and tongue, and still much was left.
			Seventh day	Very few micrococci free and in chains, in blood.	Diminution of white blood corpuscles; no loculated cells.	Doing well	
			Ninth day	No micrococci	Blood normal	....do	The patient recovered perfectly.

A study of the first of these tables shows that examinations were made of the blood of twenty-one cases of diphtheria in Philadelphia, and that in eleven of these cases no organisms were found at all, whilst in ten cases micrococci were detected in more or less abundance during life or directly after death.

A further study also shows that but one case of the eleven without micrococci died, and that in this case (Case 4) the cause of death was laryngeal stenosis, produced by the local disease, and no autopsy was permitted. The experience in Case 2 shows that it is possible to fail in the detection of micrococci, although they may exist in the blood which is in the central organs, for a very careful examination of the blood in the finger, made by both of us conjointly, did not reveal the existence of micrococci which the autopsy, only thirty-two hours later, showed to be in advanced development in the blood. It is therefore very probable that if an autopsy had been permitted in Case 4 organisms would have been detected. Of the ten cases in which fungal organisms were found six died.

When the epidemic cases are scrutinized it will be perceived that, of the Ludington cases, micrococci were present in six cases and absent in four cases, the latter all very mild and in one family. Case 26 is not considered, as the child was convalescent at the time of examination.

Of the Lakeview cases, in only seven were micrococci found in the blood, whilst in ten cases no micrococci were apparent. In regard to the mortality, we have no knowledge of a fatal termination in more than two cases, but it is very plain that the cases in which no micrococci were present were much less severe than those in which the organisms were detected.

The observations made in Philadelphia, Ludington, and Lakeview are therefore in accord. In each instance, the cases in which there were no micrococci were nearly all of them light or in the stage of convalescence, and the amount of the fungi present in the malignant cases seemed to be proportionate to the severity of the constitutional symptoms, and to steadily progress with the disease in the fatal cases.

The study of these cases is sufficient to enable us to formulate as established the proposition that *in the endemic diphtheria micrococci are always present in the part locally diseased, but are usually not present during life in the blood or in the glandular organs, even in cases which prove fatal from the interference with the breathing by the local disease;* that in *epidemic diphtheria micrococci are always present in the part locally diseased, and are also usually, and perhaps always, to be found in the blood and tissues of severe cases with marked constitutional symptoms, but are frequently, if not usually, absent from the blood of mild cases;* that *the difference is in degree, not kind, as the micrococci are usually present in the blood of malignant cases, whether of endemic or epidemic origin.*

At first thought this proposition might be considered to prove that bacteria are not an essential but an accidental phenomenon of diphtheria. We think, however, that this is hardly a warranted deduction. The micrococci are always present in the throat, and are probably also always present when very marked constitutional symptoms occur. It must be borne in mind that the distinction we have made between epidemic and endemic diphtheria is a purely arbitrary one, for convenience, and in either instance the disease may be mild or malignant, and that in the two classes of cases the symptoms are precisely parallel and the result identical.

CHAPTER III.

THE NATURAL HISTORY OF MICROCOCCI.

In the present chapter we propose to discuss the methods of recognition of micrococci, their life-history within the body, and the methods and results of their culture.

I.—Morphology of the Micrococci.

A.—*Recognition of micrococci.*

Great difficulty is sometimes encountered in distinguishing bacteria from other minute organic and inorganic particles. Certain mycelial threads and vibrios, coagulated fibrin, and protoplasmic and fat molecules often closely resemble micrococci.

As this is a question of importance, we will briefly enumerate the various methods which we have employed and found serviceable in the recognition of micrococci.

In zooglæa forms the micrococci can be distinguished at one glance by an experienced eye, as the micrococci of an individual zooglæa mass are always uniformly of one size and are always at the same distance from one another, in contradistinction to albuminous and fat molecules, which vary in size and are at varying distances from one another.

Isolated micrococci may vary in size, as may also the micrococci in the different zooglæa groups, some of the latter being made up of smaller and others of larger micrococci.

Micrococci may arrange themselves in pairs and in chains; non-living molecules or granules cannot.

Micrococci are not soluble in strong acids and alkalies, nor in alcohol and ether, as are fat and most other molecules.

Micrococci take the staining well, particularly with hæmotoxylin and aniline dyes, and the aniline dye cannot so easily be washed out as in the case of animal tissues. For this reason sections of tissues may readily be prepared in which only the bacteria are stained. Beautiful preparations may also be made by simply treating sections with a mixture of glycerine and acetic acid; the micrococci in such preparations become yellowish brown and prominent amid the perfectly translucent structures.

Tincture of iodine has proved in our hands an excellent staining fluid for bacteria within tissues. If a section has first been treated with a solution of caustic potash, iodine stains the micrococci deep yellow, while the rest of the tissue is only slightly tinged.

The most important distinguishing point between micrococci on one hand and organic and inorganic particles on the other hand is obtained by culture. Micrococci will always multiply, though at some times much more rapidly than at others. They elongate, divide, form chains or zooglæa masses if brought into a suitable culture medium.

If the above-mentioned distinctive points are observed, mistakes in recognizing micrococci and other bacteria are entirely out of the question.

In regard to the question of magnifying powers used in recognition of micrococci, we can affirm (contrary to statements of some other investigators) that a good one-fifth objective is sufficient for the detection of the diphtheritic micrococci, particularly when appropriate staining is used. We have seen no micrococci with a magnifying power of 1,500 which we could not detect with an amplification of 500 diameters. For the finer morphological details of micrococci a high magnifying power is required, and we have habitually used a one-twelfth Zeiss Homog-Immerson.

We desire also here to state that as the result of much experience we are positive that the excessive precautions often insisted upon by authors as necessary in looking for micrococci in the blood, &c., are not in fact necessary. Micrococci do not exist in the air to any great extent, and exposure of blood slides for a few minutes to the air is never followed by the appearance of micrococci when none have previously existed. This is not true of moving bacteria, which in their early growth we ourselves have in some of our former researches mistaken for micrococci, and which may appear rapidly in exposed blood. In order, however, to disarm criticism, we have in all our blood examinations used the utmost precautions.

B.—*Morphology of the micrococci as observed in cases of epidemic diphtheria, in the local cases of sporadic diphtheria, and in induced diphtheria in animals.*

When a diphtheritic false membrane was examined (by teasing it in glycerine or fresh aqueous humor) under the microscope, immediately after its removal from the pharynx, or the air passages, there was seen, besides the cellular constituents, only one kind of bacteria, viz, micrococci.

Diphtheritic membrane removed some hours previous to examination or removed *post mortem*, in addition to micrococci, yielded other forms of bacteria as described by authorities. Unless the diphtheritic alteration becomes of gangrenous character, we are not able to detect any other bacteria than micrococci. Eberth, who describes only rod bacteria in diphtheria, or those investigators who describe different varieties and mycelium of bacteria, probably examined only membranes removed some time previously, or such as were removed *post mortem*.

Our experience with a large number of cases is so absolute that we believe the occurrence of rod bacteria is proof that a membrane has begun to putrefy. It may be that in endemic or mild diphtheria the micrococci have not the power of growth that they have in malignant diphtheria, and that consequently rod bacteria have a better chance to develop than in the membrane of the malignant disease.

The micrococci were in every case of at least two sizes, according to their stage of development, or to earlier or later generation (see culture accounts). The smaller ones mostly infested leucocytes and mucous corpuscles, within which they were seen dancing in a trembling motion (the appearance has been well compared with flies caught in a fly-trap). The larger micrococci were nearly all in zooglæa form, or *infesting and apparently destroying* the epithelial cells. The latter fact is *not* in accordance with the observation of Klebs and Eppinger, who assert that the micrococci in diphtheria never infest the epithelium, but merely penetrate between the cells.

In the blood of patients suffering from the epidemic diphtheria at Ludington we always found micrococci within the white blood corpuscles (never the red ones), or in zooglæa form or to some extent free, just as they exist in the membrane. Notably the quantity of micrococci was always in the direct proportion to the intensity and the stage of the disease, as recorded in the first chapter of this memoir.

C.—*Methods and objects of culture.*

We have employed two distinct methods of culture. The first is that described by Dr. E. Klein, of London, in his report on infectious pneumo-enteritis, from whose paper the following cut is abstracted:

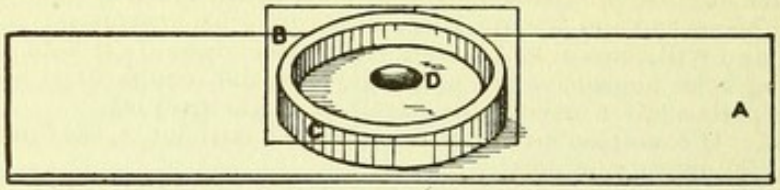

FIG. 9.

Figure I represents, of natural size, one of the culture cells.

A is the object-glass; B is the covering-glass, fixed by means of a thin layer of oil upon the upper (polished) surface of the glass ring C, which is cemented on the object-glass. The covering-glass, which should be as thin as is obtainable, has on its lower surface the droplet D, in which the fungus has been planted. In using this method the minutest speck of the infected material is removed with the point of a clean (previously heated) needle, and placed in a drop of fresh aqueous humor of the eye of a healthy rabbit, or some thoroughly sterilized culture fluid, on a thin square glass-slip. This is inverted and fixed, drop downwards, by means of a thin layer of pure sweet-oil on the glass ring.

The preparation thus mounted was placed in the incubator or oven, where the temperature was automatically maintained at various points, not below 32° and not above 45° C. In most cases we used a temperature of 37° C.

After twenty-four hours the specimen, which we will call the first generation, is used to establish a second generation in a new drop of culture fluid in the following manner: The covering-glass of the above "cell-specimen" is lifted up, and, with the aid of a fine capillary tube, a minutest quantity is removed from the edge of the droplet. In the accompanying Figure II this capillary tube is portrayed in its natural size; the contents of the tube between *a* and *b* represent about the quantity that is removed from the drop D of the previous specimen. The capillary tube is then filled with culture fluid up to about the mark *c*. A new clean covering-glass is taken; on its center is deposited a droplet of fluid from our capillary tube, and the covering-glass is then inverted and fixed with a thin layer of oil on the glass ring of another "cell." This new preparation, which represents the second generation, is also placed in the incubator and kept there at the above-named temperature for twenty-four hours. After this time it is used to inoculate another "cell-specimen," representing the third generation, and so on, from generation to generation, until the end of the culture.

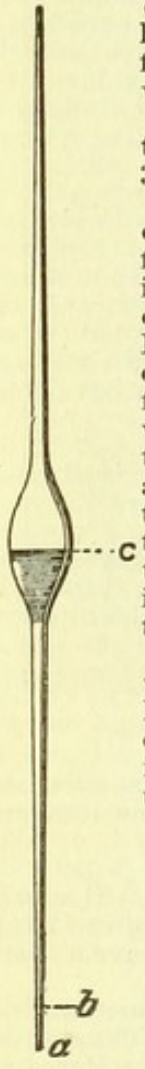

FIG. 10.

The second method employed was that perfected by Dr. Sternberg, which we believe has been already described by him in the publications of the National Board of Health. This plan has the advantage over the method of Klein that the cultures can be made on a larger scale for the purpose of inoculation of animals, but we have found the cell and glass cover more useful in observing the growth and development of the fungus.

The following objects were put in cultivation:

Sporadic diphtheritic pseudo-membrane, fresh and dried.
Epidemic diphtheritic pseudo-membrane, fresh and dried.
Fragments of spleen and kidney from epidemic diphtheria.
Pseudo-membrane from artificial croup, produced by ammonia.
Blood from diphtheria.
Blood from septicæmia.
Blood from scarlatina.
Gangrenous matter from foot.
Scrapings from tongue and mouth of normal individuals.

As a medium for the culture of the diphtheritic fungi the following fluids were used with the best results: Bouillon of chicken or rabbit, perfectly sterilized by protracted boiling; ascitic fluid whenever there was opportunity to put it in sealed tubes immediately after tapping; aqueous humor, which is drawn from living animals by a little sharp-pointed pipette, as represented in Figure II, with great facility. Other culture mediums, such as solution of gelatine, egg albumen, Pasteur's fluid, solution of dextrine, and simple syrup, were also tried, but with less satisfactory results.

As control experiments the culture fluids were usually tested by placing sealed cells in the incubator for twenty-four hours, but no organisms developed in successful cells or tubes.

D.—*Biology of the diphtheritic micrococci as observed in culture.*

If a minute particle of fresh diphtheritic matter or some scraping from tongue be put into a drop of pure culture fluid in a cell fixed in a manner as described, there are seen under the microscope a few epithelial scales infested by micrococci averaging in size $\frac{1}{28000}$, but sometimes reaching a maximum of $\frac{1}{14000}$ of an inch, and a few leucocytes, filled with minute micrococci, which, on account of their extremely small size and their trembling motion within the cell, cannot be measured; the surrounding liquid is clear, and will remain so for from six to twelve hours if kept in a cold room; but after having been placed for one single hour in the incubator at a temperature of 40° C., slight clouds appear around the particles in the droplet.

Observed under the microscope and watched for several hours, the micrococci are seen to undergo the following changes:

The micrococci contained in the leucocyte after a period of slight but very active movement, during which they look as if they were eating up their foster nurse, pass into a quiet state. They now completely fill the corpuscle, which appears to burst, and the whole internal mass escapes as an irregular glass-like body, full of quiet uniform micrococci of about $\frac{1}{30000}$ of an inch in diameter, the whole constituting the so-called "zooglæa masses." Often the collapsed vesicle was seen lying alongside of such a mass just escaped from it.

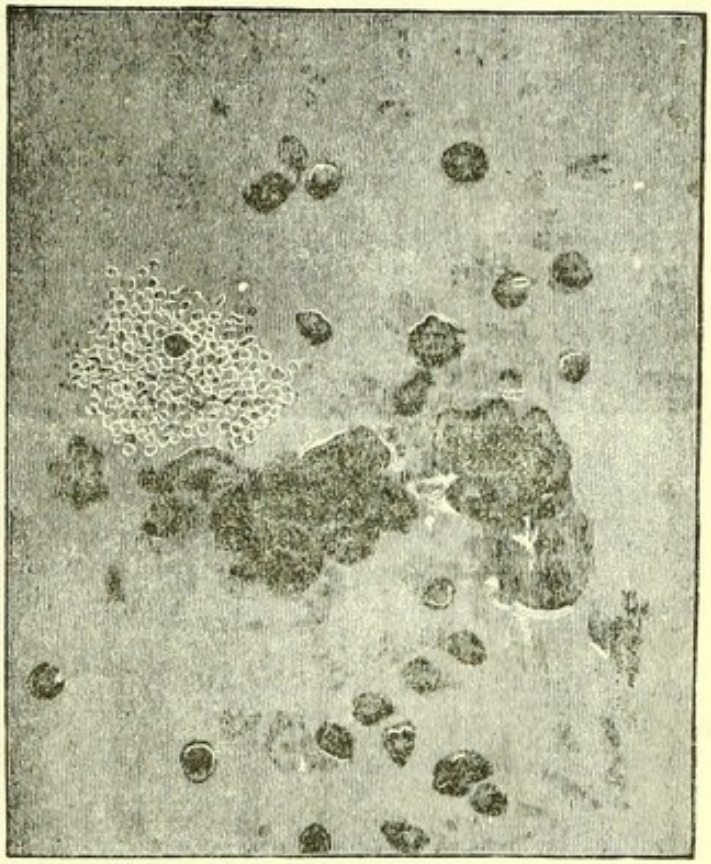

FIG. 11.—Micrococci from blood in diphtheria × 1,000, showing aureole.

The finer micrococci and those infesting the epithelial cells have grown larger and acquired a faint yellowish-green color. Some of them can always be seen to be undergoing the process of division, which is better studied, however, a little later.

After twenty-four hours the zooglæa masses can be seen to be breaking up and setting free small individual micrococci. The larger micrococci have in the meanwhile elongated; some of them resemble in appearance short rod bacteria, whilst others of them are constricted in the middle like the Arabic figure 8. The latter forms have a peculiar trembling motion, as if they were making an effort to separate the two parts. Except in the case of the peculiar vibration already noted inside of the leucocytes and this trembling before division, we have found the micrococci always motionless.

The aureole of mucous substance surrounding the isolated micrococci, particularly well described by Pasteur and Sternberg, is well marked at this stage, and is a mark of a stage of development not of a specific form. In color the larger micrococci are somewhat greenish-yellow, while the smaller ones are colorless.

After the lapse of forty-eight hours multiplication is seen to have continued, and to be occurring in micrococci originally derived from the balls. A large number of micrococci are observed elongated or in pairs, and also a few are arranged in fours, so as to resemble a sarcina, and in chains. After this no further multiplication will occur, unless new culture pabulum be added. In a second generation the multiplication will proceed in a very vigorous manner, so that within twenty-four to forty-eight hours a fresh droplet of culture fluid to which only a few micrococci have been added on a pin is completely filled out with millions of mostly round micrococci. The different forms of grouping are best expressed on the second and third day of culture. Vigorous multiplication continues also in the third and fourth generations, and occasionally in the fifth, but in later generations up to the eighth the rapidity of multiplication gradually subsides, and is hardly perceptible in a ninth or tenth generation. It is said by some authorities that micrococci kept for a while in a culture tube

will diminish in quantity and even disappear; we cannot affirm this from our own experiments. Micrococci may cease to multiply, but in many instances a diminution in their quantity had not been perceptible even after the lapse of months.

The above history refers to micrococci from true malignant diphtheria; in the case of cultures of tongue-scrapings the micrococci ceased to multiply as early as the third generation, and frequently in the second generation their growth was very slow. In addition, in tongue-scraping, some leptothrix threads frequently accompanied the micrococci in the first and second culture, and, as also as in diphtheritic culture, bacterium termo made occasionally its appearance in early generations, but *it promptly disappeared if the temperature in the incubator went for a while to* 40° and did not afterwards sink below 35°. Later generations in successful cultivation remained always clean in well-regulated temperatures and never showed any kind of mycelial fungi. Cultures with sporadic Philadelphia diphtheria gave results very similar to those achieved with tongue-scrapings; the micrococci possessed some but not very much more vitality or proliferating power, their growth usually ceasing in the fourth generation.

In regard to temperature we can affirm from our own culture experiments the following interesting facts with special reference to diphtheritic micrococci.

The micrococci multiply best at from 37° to 40° C.; higher temperatures up to 70° C. do not destroy them, but only partly arrest their multiplication, which returns, however, if the 70° is again reduced to 40° or 35°.

Fungi other than micrococci, if met with as an admixture in our culture, perished at a temperature above 50°, and proliferated well only at 30° C. and below. Too low temperature frequently spoiled our cultures, for if any spores of rod bacteria were present they usually developed and displaced the micrococci. In high temperatures, however, the micrococci are stronger and displace easily the several kinds of bacteria we observed in impure cultures.

The diphtheritic micrococci do not seem to need a large supply of oxygen for their growth; they even appear to multiply better when distant from surfaces, in contradistinction from the bacteria of putrefaction.

In culture tubes the diphtheritic micrococci are always seen as a precipitate. If there is any mycoderma at the surface it is always a sign that the culture is impure, since the bacteria found on the upper surface are always rod bacteria and bacilli, with very few micrococci. This property renders the micrococci especially fitted to flourish in dense membrane and in deep tissue, where the supply of oxygen must be very small.

In the following tables are given, in sufficient detail, the several series of cultivations which we have made.

II.—Culture Experiments.

First Series.

Diphtheritic False Membrane from the Cases of Dr. H. Allport.

Culture experiment, representing Nos. 1 to 20.

MICROCOCCI.—[Matter used: fresh diphtheritic pseudo-membrane. Case of Dr. Allport.]

Date.	No. of generations.	Culture fluid.	Mode of culture.	Temperature in incubator.	Size of.	Quantity and rate of multiplication.	Mode of grouping.				
							Isolated.	In pairs.	Sarcina form.	Torula chains.	Zooglaea.
During April and May, 1881	First	Aqueous humor of rabbit.	Cells	35° to 40° C.	$\frac{1}{25000}$ to $\frac{1}{25000}$ of an inch.	At first slow, then rapid.	Many	Few	Few	Few	Many.
	Second	do	do	do	do	Rapid	do	Many	do	do	Do.
	Third	do	do	do	do	Very rapid	do	do	do	Many	Do.

Many micrococci were in huge clouds without any special of form grouping.

All the cultures of this series yielded crops of pure micrococci. No other bacteria interfered in successful tubes. Mycelial fungi were never observed.

SECOND SERIES.

Endemic diphtheritic matter obtained from time to time in Philadelphia.—Twenty-five cultures were made, and all the mentioned culture fluids were tried in this series up to a fourth generation. The comparative values of the culture fluids were referred to before.

The general results of the culture experiment were similar to those of the first series up to the second generation. In the third generation the micrococci already began to fail in growth and multiplication, and the fourth generation yielded a still poorer crop. The cultures were occasionally impure—the rod bacteria supervened.

The regulation of the temperature was sometimes defective during the experimentation with this series on account of imperfect working of the mechanism of the incubator. Sometimes the temperature fell down to 20° during the night, and it was frequently observed that rod bacteria made promptly their appearance in large numbers during such intervals and spoiled the cultures. The same occurred with clean cultures when heat was suspended purposely for several days. Those culture tubes, however, which were subjected to a uniform temperature of not below 37° C. did not show any invasion of rod bacteria, and the growth of micrococci continued in the manner described.

The careful measurement of the micrococci in this series, which was best accomplished by measuring the chains of micrococci, revealed a size of $\frac{1}{14000}$ to $\frac{1}{12000}$ of an inch in diameter, the first size relating more to micrococci in zooglæa form. The torula chains were usually made up of the smallest micrococci, approaching in this respect to those of the micrococci balls which we have proven to be formed by the invasion of leucocytes by micrococci.

THIRD SERIES.

Thirty cultures were made with matter other than diphtheritic, being carried on to the fourth generation. Although the matter used was of the most diverse character, such as ammonia croup (croupous exudation produced in the trachea of a rabbit by ammonia), slough from gangrene of foot, yet the results in culture were nearly all identical.

One and the same thing gave at one time an excellent crop of micrococci if the temperature of the incubator was kept up high enough and pains were taken to keep the culture pure; whilst at another time with the same culture fluid the same tube showed only a few micrococci, but rod bacteria in abundance; this could nearly always be traced to low and irregular temperature of the incubator. In some cases after the replacing of the micrococci by rod bacteria, the tubes were subjected to a high temperature for several days, when the bacteria disappeared, whilst the micrococci did not reappear, so that the liquid was left clear. But even in successful cultures the growth and multiplication of micrococci were observed to be prosperous only to the second or very rarely to the third generation. In later generations the fungi grew scantily.

Micrococci from epidemic diphtheria, fifth generation × 1,000.

In size, shape, and color, as well as in the mode of grouping, the micrococci were the same as those from the diphtheritic pseudomembrane. If this were not the case in one generation, it invariably was so in the next.

FOURTH SERIES.

Twenty-five cultures were made with epidemic diphtheritic matter from Ludington, and were carried on to the fifth generation. The results were identical in every one of the successful cultures, and the record of one experiment holds good for all the rest.

Neither rod-bacteria nor mycelium could be detected at any time of culture. One of the culture tubes did not show any change in appearances when examined two months later, it having been kept during the whole time in the incubator.

Culture experiment, representing Nos. 76 to 100.

MICROCOCCI.—[Matter used: dried diphtheritic membrane. Case of Dr. Shorts, Ludington.]

Date.	No. of generation.	Culture fluid.	Mode of culture.	Temperature in incubator.	Mycoderma or precipitate in tube.	Size of.	Quantity and rate of multiplication.	Mode of grouping.				
								Isolated.	In pairs.	Sarcina form.	Torula chains.	Zooglaea.
During July, 1881....	First	Bouillon or ascitic fluid.	Cell and tube.	37° C.	Precipitate.	$\frac{1}{35000}$ to $\frac{1}{12000}$	Slow; twelve hours later slow but marked.	Few.	None.	None.	None.	Moderate quantity.
	Second, next day.....	...do	...do	[illegible]	....do	....do	Faster; ten hours later rapid.	Many.	Many.	Some.	None.	Large quantity.
	Third, third day.....	...do	...do ...	38	....do	....do	Rapid.....	Large quantity in all forms of grouping.				
	Fourth day.....	...do	...do	40	....do	....do	Enormous	Cell specimen is so crowded that mode of grouping is indistinguishable.				
	Fourth, fifth day.....	...do	...do	39	....do	....do	Rapid.....	In all forms.				
	Fifth, seventh day...	...do	...do	38	....do	....do	Slower	In all forms, mostly torula chains and zooglaea forms of yellowish color.				

FIFTH SERIES.

Forty cultures were made with epidemic diphtheritic false membrane received from Ludington per express, and were carried to the tenth generation. The membrane was in a semi-dry condition, and showed under the microscope the following morphological elements:

Epithelial scales and leucocytes, both infested by clouds of micrococci. The micrococci were of two sizes, larger ones of a greenish color, measuring about $\frac{1}{14000}$ of an inch in diameter, and smaller ones not exceeding $\frac{1}{35000}$ of an inch. There were also seen some rod-bacteria and bacilli, and also a number of bundles of mycelial threads, resembling leptothrix, of greenish color, united together at one end, but separated at the other. These extraneous organisms disappeared, however, in proper culture.

Particular pains was taken to have always a uniform temperature in the incubator and not exceeding 37° C. The results obtained were nearly identical with those in the fourth series. Only these cultures were conducted up to the tenth generation, and a larger proportion of impure culture were encountered here, mostly dependent upon thermal conditions. The record of one of the many uniform and successful culture experiments reads as follows:

Culture experiment, representing Nos. 101 to 140.

MICROCOCCI.—[Matter used: diphtheritic membrane removed five days previous by Dr. Shorts, of Ludington.]

Date.	No. of generation.	Culture fluid.	Mode of culture.	Temperature in incubator.	Size of.	Quantity and rate of multiplication.	Mode of grouping.					
							Isolated.	In parts.	Sarcina form.	Torula chains	Zooglaca.	Rod bacteria.
July 23	First	Bouillon of rabbit.	Cells and tubes.	45° C. (with the object to kill the extraneous fungi).	$\frac{1}{35000}$ to $\frac{1}{14000}$	Slight	Many.	A few.	None.	None.	Some.	A few.
					..do ...	Rapid	..do ...	Many.	Some.	..do ...	Large quantity.	None.
24	Second	... do	....do	37								
25	Third	... do	....do	37	..do ...	Enormous	..do ...	..do ...	..do ...	..do ...	..do ...	Do.
26	Fourth	... do	....do	37	..do ...	Rapid	..do ...	..do ...	..do ...	Many.	..do ...	
28	Fifth	....do	....do	37	..do ...	Slower	..do ...	..do ...	..do ...	..do ...	..do ...	
29	Sixth	....do	....do	37	..do ...	Slow	..do ...	..do ...	do ...	..do ...	..do ...	
31	Seventh	....do	....do	37	..do ...	....do	Only isolated and in torula form.					
Aug. 2	Eighth	... do	... do	37	..do ...	Very slow	Do.					
4	Ninth	....do	....do	37	..do ...	Hardly perceptible, or not at all.	Do.					
7	Tenth	... do	....do	37	..do ...	....do	Do.					

Neither mycelium nor rod-bacteria were observed in successful cultures, except in the early (first and second) generation.

SIXTH SERIES.

Cultivation of tongue-scraping.—To test the behavior of the micrococci from the mucous membrane of the tongue in cultures, five experiments were made and carried to the fourth generation. In every experiment a result was obtained that the proliferating power of these micrococci was very weak in comparison with those of the diphtheritic exudate. Only in the second generation they multiplied rapidly and precisely in the same manner as the diphtheritic micrococci, and in this generation they are morphologically perfectly identical with them.

SEVENTH SERIES.

Five cultures were made with epidemic diphtheritic matter which had been for two months exposed to air and light, frequently to the direct rays of the sun. The results of the culture were surprising, as it was found that the micrococci of this dried membrane had lost a great deal in their proliferating power. In the second generation a moderate crop of micrococci were obtained, whilst in the third generation very slow or hardly any multiplying property could be observed.

This shows distinctly that the same diphtheritic micrococci which in the fourth series of cultures had a vigorous power of growth and multiplication had lost greatly these properties (simultaneously with the loss of virulence upon animals).

CHAPTER IV.

ON THE RELATIONS OF THE MICROCOCCI TO DIPHTHERIA.

In the present chapter we propose considering the rôle which micrococci play in diphtheria. Before detailing our own work, it seems but proper to give a brief *résume* of that which has been done by others. We shall do this under two headings.

SECTION A.—*BIBLIOGRAPHICAL.**

(I.) IN FAVOR OF THE FUNGOID ORIGIN OF DIPHTHERIA.

OERTEL (*Studien über Diphtherie Aerztl. Intelligenzblatt*, 1868, No. 31) was the first to speak of micrococci as occurring in diphtheritic pseudo-membrane, and also in the blood, the lymphatic vessels, and the kidneys of persons affected by diphtheria. He describes them as "point-like, dark-contoured, round or oval (immovable)" little bodies, occurring either isolated or in zooglæa form. Later (*Deutsches Archiv f. klin. Medicin*, 1871, p. 242, and in *Ziemssen's Encyclopædia*, 1874), Oertel describes the diphtheritic fungi to be spherical bacteria (micrococci, accompanied by a larger or smaller quantity of bacterium termo). He thinks that they are the direct cause of the diphtheritic inflammation; that long before any false membrane is formed they attack the mucous membrane and induce the disease.

Oertel experimented as follows:

First series.—Produced ammonia croup in rabbits; found few or no micrococci in the false membrane, and never any in the blood and organs. Hence he thinks that croup must be strongly separated from diphtheria.

Second series.—He inoculated with true diphtheritic matter in the trachea, twelve rabbits, three pigeons, and two chickens; and produced local lesions and constitutional disturbances perfectly identical with those of diphtheria in man; micrococci were found abundant in the blood of the poisoned animals.

Third series.—He inoculated rabbits with true diphtheritic matter, placing the poison below the skin and in the muscles, with the same results as in the second series

Fourth series.—He inoculated rabbits with pyæmic pus and gangrenous tissues with negative results.

Fifth series.—He succeeded by inoculations in the trachea in transmitting the disease—

1st. From man to rabbit No. 1.
2d. From rabbit No. 1 to rabbit No. 2.
3d. From rabbit No. 2 to rabbit No. 3.
4th. From rabbit No. 3 to a pigeon.
5th. From the pigeon to rabbit No. 4.

All the animals died with lesions peculiar to diphtheria within thirty to forty hours. How the bacteria acts he says is not known.

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* This paper was sent by its authors to the National Board of Health in September, 1882; of course literature of a later date is not considered.

Oertel further affirms that the quantity of fungi present in any case bears a direct relation to the intensity of the morbid process; they multiply as the disease advances and diminish with its retreat. Also, that the special form of fungus is never present in simple inflammation of the fauces or in mercurial stomatitis; but that, if the diphtheritic process intervenes on these disorders, the fungus at once makes its appearance, and quickly displaces the more common forms of bacteria which are present.

NASSILOFF (*Virchow's Archiv*, vol. 50, 1870), from his observations and experiments, came to the conclusion that the fungi found in diphtheria are identical with those occuring in decomposing animal tissues. His conclusions are:

(1.) Fungi are always present in diphtheritic membranes.

(2.) The development of the fungi precedes the formation of the membrane, and the fungi are the direct cause of the necrotic changes in diphtheritic inflammations.

(3.) The fungi penetrate into the tissues by way of the juice channels and lymphatics before any changes in those tissues can be observed, so that fungi are the cause of the inflammation and the destruction of the parts affected.

HUETER AND TOMASSI (*Med. Central-Blatt*, 1868, Nos. 12 and 34), also established by experiment the direct causative relation of micrococci to diphtheria. They placed particles of diphtheritic membranes in the muscles of the back of rabbits. Death followed promptly within forty hours. The tissues at the seat of inoculation were inflamed and inves ed by very small, roundish micrococci. The latter were found also during life in the blood of the infected animals.

TRENDELENBURG (*Arch. f. klin. Chirurgie*, 1869, vol. 10) put diphtheritic matter in the trachea of rabbits and pigeons. He succeeded in producing false membrane only in eleven out of sixty-eight experiments; in the blood of rabbits inoculated with diphtheritic matter he frequently found micrococcci, sometimes even in those animals in which a distinct false membrane had failed to appear. Frequently, but not always, he found micrococci in the blood of children suffering from the disease.

Trendelenberg doubts whether the micrococci as found in the blood of diphtheritic patients are peculiar to diphtheria, and does not believe it to be proof of the transmission of diphtheria to animals if at the seat of inoculation a diphtheritic inflammation is produced.

EBERTH (*Correspondenz-Blatt der schweizer Aerzte*, No. 1, 1872) discovered in 1871 micrococci in the blood and the tissues of persons who had died of diphtheria, but was not able to detect the fungi in the blood or even the false membrane of patients suffering from true croup.

Experimentally he succeeded in reproducing diphtheria in animals, which died in three days.

Diphtheria of wounds, Eberth thinks, stands in close relation to pyæmia. Pyæmia, he says, "is a diphtheria proceeding from a wound." The bacteria of diphtheria and pyæmia he believes to be identical.

LETZERICH (*Virchow's Archiv*, 1873, vol. 58) claims to have produced local and general diphtheritic lesions with the fungi filtered out from the urine of individuals suffering from that disease. When the dried urine filter, with the pure fungi on it (all other foreign matter having been washed out by means of hot water), were introduced into the uninjured mouths or vaginas of rabbits, diphtheritic pseudo-membranes and death followed in from three to five days.

He describes the fungus as occurring in four forms.

First. Constituting small balls composed of a hyaline substance, which is colored blue by iodine and sulphuric acid, and of small punctiform roundish or elliptical little bodies, which may increase until the whole mass becomes very dark.

Second. Large balls "*Plasmakugeln*" with a seeming outer membrane.

Third. Large irregular masses formed by the bursting of these balls.

Fourth. True mycelial filaments which produce spores of a tilletia, and are named by Letzerich *Tilletia diphtheritica*.

COHN (*Beiträge zur Biologie der Pflanzen*, 1872 and 1873), who regards bacteria as algæ, classes the diphtheritic plant with the schizophytes; tribe, sphæro bacteria, and calls it *Micrococcus diphtheriticus*. He gives as its specific character: Perfectly round, less than one micromillimeter in size; in multiplying, first forms in pairs, then several joint, then chains, and finally zooglæa masses, but never any mycelium.

KLEBS (*Correspondenz-Blatt der schweizer Aerzte*, 1871, No. 9) thinks that micrococci do develop a *mycelium* resembling *Leptothrix buccallis*, but somewhat thinner or more delicate than it is. This diphtheritic mycelium forms on the surface of affected membranes dense bunches upon which spores are produced. The latter collect in masses in zooglæa form of various size and shape. Between these groups occur free micrococci ("*Monades*"), micrococci chains, and rod bacteria. The greatest destruction of tissues is produced by the zooglæa.

Klebs agrees with Eberth concerning the identity of diphtheritic and septic micrococci. In his latest publication (*Archiv für experiment. Pathologie und Therap.*, vol. 4, p. 191), Klebs describes the diphtheritic fungi as follows:

First stage.—Very small micrococci forming in the quiescent state sharply defined

round balls, the individual micrococci being united in rows or chains and imbedded in a very scanty gelatinous matter.

Second stage.—Peripheral layers of the micrococci balls give rise to mobile bacteria, which do not need much oxygen, and have a tendency to penetrate into and live within the tissues and juices where not much oxygen is present.

Third stage.—The highest form of development consists in the formation of a mycelium in the tissues. The threads are never branching, and arrange themselves parallel to one another, covering the outer surfaces like the grass in the turf. Occasionally mycelium masses may form in deeper structures of the organisms.

Professor Klebs also gives the following as peculiarities in the life-history of the diphtheritic fungus:

(*a.*) They can grow and develop without oxygen.

(*b.*) They are killed only by a temperature of 65° to 70° C. (Not below that.)

(*c.*) In themselves they do not develop any fœtid gases.

(*d.*) They are sometimes of intensely yellow color.

(*e.*) They may circulate in the blood, but preferably are deposited outside the blood-vesssels, where they may form mycelial masses.

They never penetrate into the substances of the epithelial cells, but go between them (Klebs and Eppinger). They afford a distinction between diphtheritic and inflammatory croup.

M. Ch. Talamon describes a fungus as the true diphtheritic poison, which has a remarkable resemblance to a penicillum. We quote from a translation in the *Chicago Medical Examiner*. He says he has cultivated it in damp cells, and, that after its evolution is completed, it appears under the shape of mycelia and characteristic spores. These mycelia sometimes appear as long tubes, partitioned from distance to distance, specially refracting the light, and generally very clear; they are from 2 to 4 and 5 thousandths of a millimeter wide (.000002 to .000005). When the fluids are well conditioned they stretch extremely, at times branch off, and these divisions are themselves peculiar; they form by their slightly incurved branches a figure best compared to a lyre or a tuning fork. At other times the mycelia do not stretch in this way, although multiplying so as to rapidly cover the surface of the liquid of cultivation, they remain short, take on odd shapes, the most common of which resemble a crutch; there is always present, then, a multitude of straight cylinders, 4 or 5 thousandths of a millimeter wide by 15, 20, or 40 thousandths long (.000004^{m} by .00005^{m} or .00004^{m}).

There are two kinds of spores—round or oval—which may be considered the spores of germination, and rectangular spores which represent the last phase of growth of the fungus, and which we will call conidia (conidies). These give the species its particular character; their shape is rectangular and their size very liable to vary; their width varies from m. .000005 to .000015. Sometimes they are isolated, other times they are in groups of 2 or 3; often they form beads of 10, 12, or 15 individuals, or a small chain in zigzag. Homogeneous at first, they soon become filled with small, round, bodies, very brilliant, the size of common micrococci, which appear to me as the true germ of the fungus.

The round or oval spores form the mycelium by stretching; they look like bright specks m. .000003 to .00005 in diameter, in the middle of granular matter, spread in sheets of greater or lesser extent, which represent what is called zooglea. The spores stretch one of their poles so as to form a tube m. .000002 to .000004 in diameter which branches as above stated. When the lengthening of the mycelium is first begun, the space with its prolongation represents a tadpole. Mr. Talamon claims to have produced diptheria in rabbits, guinea pigs, cocks, and pigeons by inoculating with this fungus, and even some frogs who were fed upon the fungus promptly succumbed to the disease.

(II.) AGAINST A FUNGOID ORIGIN OF DIPHTHERIA.

Senator (*Berliner Klin. Wochenschrift*, 1872, Nos. 26, 27, 31, *and* 33, and *Volkmann's Sammlung klin. Vorträge*, 1874, No. 78) declares himself decidedly against a fungoid origin of diphtheria, on the following grounds:

Other diseases of the mouth and the pharynx are accompanied by the same micrococci which are also found in the mucus between the teeth and in the urine of the normal man, micrococci being only the spores of *Leptothrix buccallis*. The diphtheritic micrococci do not differ in cultures in any way from those of other origin.

Experimental proof.—Inoculations with any putrefying substances have given the same local and secondary changes as those produced by true diphtheritic material.

Scarlet fever produces precisely identical local lesions in pharynx as those which occur in diphtheria.

Micrococci multiply enormously on account of the favorable conditions in the pharynx created by the deep-seated ulceration; when ulceration is absent, as in the larynx, the micrococci are in only very moderate number; probably the carbonic acid is exerting also some antiseptic effect in the latter case.

BILLROTH (*Untersuchungen über Vegetationsformen der Cocco-bacteria Septica, &c.*, Berlin, 1874), who investigated the bacteria question exhaustively, came to the conclusion that they are only concomitant with diseases, and expresses himself positively against the view that the different affections are caused each by special forms of bacteria. He says that the so-called pathogenic bacteria of diseases are positively identical with those found in putrefying dead tissues.

LEBERT (*Ziemssen's Handbuch der acuten Infectionskrankheiten*, vol. 1, p. 255) is also against the fungoid theory, on the ground that nothing has yet been proven.

WAGNER thinks there is a great difference between the human pseudo-membrane and the experimentally-produced false membrane.

BEALE (Diseased germs, London, 1872) denies specific fungi altogether.

NAGELI thinks that all varieties of bacteria are derivatives of one and the same species of fungi.

Dr. JACOBI, of New York, also entirely dissents from the fungal origin of diphtheria.

Dr. C. HARLEY (Pathol. Transact., vol. x, p. 315) failed to inoculate animals with diphtheritic membrane.

In a voluminous study of the subject, Drs. SATTERTHWAITE and CURTIS (Report of Dr. Edw. Curtis and Thos. Satterthwaite to the New York City board of health, New York, 1877) are also opposed to the fungal theory, on account of the following facts and deductions, founded upon their own researches:

First. The bacteria of diphtheritic membranes do not differ in optical or chemical behavior from those found in putrescent but non-diphtheritic animal material.

Second. They believe the disease produced in rabbits by diphtheritic inoculation is not diphtheria, and find that "Scrapings from the upper surface of a somewhat furred tongue from a healthy person" produce, when placed in the cellular tissue of the rabbit, symptoms and results exactly similar to those caused by inoculations with diphtheritic membrane; also that inoculations of rabbits with putrid Cohn's fluid produce similar results to those.

Third. Salicylic acid added to the diphtheritic matter does not prevent its action, although, as they believe, it acts fatally upon the germs.

[This last objection is not, as it seems, a conclusive reason, for it is very possible that salicylic acid may arrest the growth of micrococci without killing them. In the experiments of Curtis and Satterthwaite it was shown that before the injection the micrococci did not grow in the presence of the salicylic acid; after the injection the salicylic acid is undoubtedly removed from the injected material by being absorbed into the system, the micrococci remaining largely at the seat of inoculation. Under these circumstances, if the micrococci were only benumbed, not killed, by the acid, they would of course soon begin to grow and assert themselves.]

SECTION B.—*ORIGINAL STUDY.*

The first series of experiments with the lower animals which we made had for their object to determine whether it is possible to produce the diphtheria in them by inoculations of diphtheritic matter. Clinically the mild form of the disease which is seen so constantly in our large cities separates itself from the malignant epidemic which occurs at intervals in various localities, although even in what may be termed endemic diphtheria malignant cases occasionally do occur. Following this clinical variation, we have separated our experiments with inoculations into those made with material obtained from cases occurring in this city when no epidemic influence was rife and those taken from cases during distinct epidemics in several localities.

SECTION A.—ENDEMIC DIPHTHERIA.

FIRST SERIES OF EXPERIMENTS.

Inoculation with diphtheritic matter subcutaneously and in the mucous membrane of the mouth.

Number of experiment.	Date of inoculation.	Animal.	Inoculation.	Recovery or death.	Result of autopsy, and of microscopic examination.	Remarks chiefly upon source of matter employed.
1	Apr. 22	Small rabbit, No. 1	Inoculated on tongue with fresh diphtheritic membrane.	Remained perfectly well to May 16, 24 days, when they were accidentally killed.	No signs of diphtheritic inflammation anywhere. The spleen, lymph glands, liver, and lung showed some tubercle granulations. The rest of the organs normal. No bacteria in the organs.	Case I.—Material taken by Dr. Cardeza from child, 3 hours previously to inoculation. Child's throat had been touched with tincture of iron and glycerine previously.
2	Apr. 22	Small rabbit, No. 2	Same as last			
3	Apr. 24	Small rabbit, No. 3	Same as last	Remained well		Same case. The throat for 24 hours previous to the removal had not been touched by anything.
4	Apr. 24	Small rabbit, No. 4	Same as last	Died May 10	No lesions except lymphatic glands swollen and tuberculous; also spleen. No bacteria in organs.	Same case, and exudation as last.
5	Apr. 24	Large gray rabbit, No. 5.	Inoculated in mouth; had also a piece of membrane put under the skin of the side.	May 4, was feverish for several days; a hard lump developed on the side at the seat of inoculation consisting of cheesy matter. June 6, animal well, cheesy lump smaller but persistent. Animal remained well.	Cheesy matter composed of pus, compound granule cells, débris, and numerous bacteria.	Same case. The child recovered from the acute attack, which was followed by paralysis, finally resulting in death.
6	Apr. 29	Small albino rabbit, No. 6.	Inoculated with fresh diphtheritic membrane in mouth, tongue, roof, and pharynx, and also subcutaneously on thigh.	Died May 2, 70 hours after inoculation, in convulsions, which commenced 6 hours previous to death.	Small cheesy lump at the seat of subcutaneous inoculation. Tongue ulcerated; no signs of diphtheritic inflammation anywhere; all organs hyperæmic, otherwise of normal appearance, but upon microscopic examination tubercular granulations well marked in lung, liver, spleen and lymphatic glands. No bacteria in organs, except lungs, where are also hemorrhagic infarctions.	Case II.—Removed by Dr. Dunmire from the throat of child a few hours previously.
7	Apr. 29	Small albino rabbit, No. 7.	Same as last	Animal remained well		Same case.

First series of experiments.—*Inoculation with diphtheritic matter subcutaneously and in the mucous membrane of the mouth*—Continued.

Number of experiment.	Date of inoculation.	Animal.	Inoculation.	Recovery or death.	Result of autopsy and microscopic examination.	Remarks.
8	Apr. 29	Small rabbit, No. 8	Same as last	Remained well to May 1, when killed accidentally.	Post mortem revealed no lesions	Case III.—From Dr. Cardeza; inoculation made 15 minutes after removal of the membrane from the child's throat. This case was in family of No. 1, and originated apparently by contagion.
9	Apr. 29	Small rabbit, No. 9	Same as last	Remained well to May 16, when accidentally killed.		
10	Apr. 29	Albino rabbit, middle sized, No. 10.	Same as last	Remained well.		
11	Apr. 30	Rabbit, No. 3	Reinoculated with diphtheritic matter in mouth and subcutaneously. May 7, inoculated third time.	Remained well up to May 13; found dead May 14.	Small cheesy lump on thigh at the place of inoculation. All organs tubercular, otherwise to the naked eye of normal appearance. No bacteria in organs.	Case IV.—From Dr. James Collins, twelfth day of disease. Matter semi-liquid, mixed with blood. The case afterward died.
12	Apr. 30	Dog, No. 1	Inoculated with diphtheritic membrane in mouth and thigh.	Remained well		Same case.
13	Apr. 30	Large cat, No. 1	Inoculated with diphtheritic matter in mouth and subcutaneously on right thigh.	Remained well; a large lump developed in skin at seat of inoculation, which eventually disappeared. No micrococci in blood.		Case IV.—From Dr. Collins. Case recovered.
14	Apr. 30	Rabbit, No. 11, large albino.	Same as last.			
15	Apr. 30	Rabbit, No. 12, large old albino.	Same as last	Remained well.		
16	May 1	Small rabbit, No. 13.	Same as last	A small cheesy lump developed at seat of inoculation; died May 12.	Slight exudation on the mucous membrane of the larynx and trachea, of grayish color, and translucent. Trachea and lungs much congested. All organs tubercular; in the lungs are seen profuse hemorrhagic infarctions, and numerous bacteria. No bacteria in the organs.	Case V.—From Dr. W. S. Stewart. Membrane inoculated fresh on the day of removal.
17	May 1	Large cat, No. 2	Same as former	Remained well; lump developed on side and disappeared within a week, healing perfectly. No micrococci in blood.		Do.
18	May 1	Small cat, No. 3	Inoculated in mouth	Remained well		Do.
19	May 1	Dog, No. 2	Inoculated in mouth, pharynx, tonsils, tongue, and also subcutaneously	Remained well. Killed June 11.	Cheesy lump which had existed for a long while on the side found to have been absorbed; in its place was a	

			on the side, with fresh diphtheritic matter. The latter also given to the animal mixed with food.		hard lump, probably of cicatricial tissue. No lesions perceptible. Blood examined during life did not show any bacteria.	
20	May 4	Small cat, No. 4 ...	Inoculated with dried diphtheritic matter in mouth and subcutaneously.	Remained well. Killed June 12.	No lesions found; blood did not contain bacteria.	Case VI.—From Dr. Frank R. Brunner. Membrane removed several days ago from woman of 45 years; apparently much decomposed.
21	May 4	Large rabbit, No. 14.	Same as in last experiment..	Remained well. Has large lump at place of skin inoculation.	No lesions except a large cheesy lump on side. No microscopic examination made.	
22	May 4	Cat, No. 5.....	Same as last	Remained well. Killed June 12.	Large cheesy lump at the place of subcutaneous inoculation; no signs of diphtheritic inflammation; lungs highly congested, the remaining organs all appearing normal to the naked eye; but microscopic examination showed tubercular granulations everywhere; hemorrhagic infarctions in lungs; no bacteria in organs except lungs.	Case VII.—From Dr. Collins.
23	May 7	Large rabbit, No. 15.	Same as last	Remained well up to May 19. Found dead May 20.		
24	May 7	Small rabbit, No. 9.	Reinoculated with fresh diphtheritic matter in mouth and subcutaneously.	Killed accidentally.....	Post mortem revealed no lesion.....	See experiment 9.
25	May 7	Small rabbit, No. 3.	Inoculated the third time as before with fresh matter.	Died May 9.....	A small cheesy lump on thigh at the place of inoculation; all organs tubercular, otherwise to the naked eye of normal appearance; no bacteria in organs.	
26	May 9	Dog, No. 3.....	Inoculated in mouth and skin with fresh membrane.	Remained well.....	.....	Case VIII.—From Dr. James Collins.
27	May 9	Small cat, No. 6				
28	May 9	Small cat, No. 7	Same as the last.....	Remained well. Killed June 12.	All had cicatrizing lump at place of skin inoculation; no other lesions detected; no bacteria in blood.	From Dr. James Collins.
29	May 9	Small cat, No. 8				
30	May 11	Goat, No. 1	Inoculated in mouth and subcutaneously with fresh membrane.	Remained well.....	.....	Case IX.—From Dr. H. C. Wood. Taken from a case the second day of the disease, the attack having been derived by contagion from the cases of Dr. Cardeza.
31	May 11	Cat, No. 9.....	Similar inoculation.....	Remained well.		
32	May 11	Cat, No. 10.....	Same as before	Found dead June 11	No microscopic examination made; specimen preserved.	

First series of experiments—*Inoculation with diphtheritic matter subcutaneously and in the mucous membrane of mouth*—Continued.

Number of experiment.	Date of inoculation.	Animal.	Place of inoculation.	Result.	Autopsy and microscopic examination.	Remarks.
33	May 24	Small black rabbit	With dry, fresh material in left thigh.	Died June 10	Blood from ear was examined May 29, and no micrococci found. Autopsy did not show anything abnormal. Death could not be accounted for. Microscopic examination of organs did not reveal micrococci in vessels of organs.	Matter taken from a fatal case (Dönges child).
34	May 24	do	do	Remained well		Do.
35	May 24	Large young rabbit	do	Died June 14	No micrococci in blood. Large cheesy lump at part of inoculation; lymphatic glands enlarged and cheesy. Tuberculosis? Nothing abnormal found.	Do.
36	May 31	Albino rabbit	With fresh, not dried, diphtheritic matter (from Dönges child) in left thigh.	Died June 4	Blood examined just before death shows blood crowded with micrococci in white blood corpuscles and free and single, and in zooglæa form. Large local pseudo-membrane.	Do.
37	May 31	Albino rabbit, young	do	Died June 5	Blood crowded with micrococci. White blood corpuscles; all loculated and full with micrococci.	Do.
38	May 31	Small gray rabbit	do	Died June 9	Some micrococci in blood, free and in white blood corpuscles and in zooglæa masses.	Do.
39	July 8, 8 a. m	Large steel-gray rabbit	Inoculated below skin of neck and into tonsils.	Found dying July 9, in the evening.	Wound of neck slightly sloughing. Autopsy immediately. Blood from jugular vein shows micrococci in nearly all white blood corpuscles and some few micrococci free. No zooglæa masses. Tonsils and pharynx much inflamed and swollen and ulcerating, but not showing definite false membrane. Trachea and lung congested. Rest of organs appear normal. Microscopic examination of tonsils, spleen, and marrow of bone showed multitude of micrococci, but none or very few could be discovered in kidney and liver.	Membrane taken at the post mortem from a child at Blockley Hospital (Dr. Kollock's case). It was about 36 hours after death, and very stinking; weather very hot.
40	July 8, 8 a. m	Large yellow-brown buck rabbit.	do	Remained well	Was ill and did not take food, but recovered on third day. Blood exam-	Do.

					ined July 9; did not show micrococci.	
41	July 8, 8 a. m ...	Middle-sized yellow rabbit.	Inoculated only below skin of left thigh.	Died July 10, afternoon.	Wound covered with false membrane. Blood contained micrococci in white blood corpuscles and free. Kidney, liver, and spleen showed micrococci emboli.	Do.
42	June 24.....	Large black and white rabbit.	Inserted about ten grains of solid, fresh diphtheritic matter below skin, back of right ear.	No result	A cheesy lump formed, but subsequently discharged and wound healed.	Diphtheritic matter fresh from a case of Dr. Montgomerry, but had been left soaking in water for 30 hours.
43	June 24.....	Large black rabbit.....	.....do	....do.....	Wound healed perfectly.....	Do.
44	June 24.....	Small gray rabbit.....	.....do	Died July 2.....	Large sloughing cheesy ulcer behind ear, right side of head, and the eye reddened and swollen, not unlike erysipelas. Tissue around lump full of micrococci and small bacilli. Blood showed micrococci in white blood corpuscles and a few zooglæa masses. Organs examined under microscope in sections for micrococci emboli, but none could be discovered.	Do.

In the table just given there are recorded forty-four experiments, in only fourteen of which the animal died unless killed accidentally or otherwise.

The time between the dates of death and of the last inoculation was: Experiment 4, six days; experiment 6, seventy hours; experiment 11, fifteen days; experiment 16, eleven days; experiment 23, thirteen days; experiment 25, two days; experiment 33, seventeen days; experiment 34, twenty-two days; experiment 36, four days; experiment 37, five days; experiment 38, nine days; experiment 39, one and a half days; experiment 41, two and a half days; experiment 44, eight days.

The question naturally arises as to whether the few animals in which the inoculation was followed by death died of diphtheria or of some other disease. Of these fatal cases there were only seven in which a strong suspicion of septic poison could fairly be entertained; death was too long delayed. In one of these, experiment 25, the animal died two days after the third inoculation, but it had been first inoculated fifty-two days previously, and was very tubercular; so that the last inoculation may have had nothing to do with the fatal result. In experiment 6, no bacteria were found except in the lungs; no false membrane existed anywhere; the lungs were full of hemorrhagic infarctions, and the peculiar, long-continued convulsions are not a symptom commonly seen in septic diseases of rabbits. There is, therefore, no proof that the rabbit died of diphtheria or of septic poisoning; it is probable that the wound of the larynx brought about the fatal result. Experiments 36, 37, 38 constitute a group of inoculations from one case in which there were very decided evidences of septicæmia in the rabbit both before and after death; the same statements apply to experiments 39, 41: omitting these two groups of fatal cases, we find that in only one of the fatal cases were there any exudations present in any organ which could give rise to the slightest suspicion that the animal died from diphtheria. In this case, experiment 16, there was only a catarrhal inflammation of trachea (with an exudation) which presented some of the characteristics of false membrane. In none of these cases were micrococci found in the blood. There is, therefore, little reason for believing that the rabbits died from diphtheria or even from septic poisoning. Of what, then, did they die?

A study of the post-mortem reports will show that in nearly every case the internal organs were tubercular, and in many cases intensely so; also, that tubercular disease was found in the organs of those rabbits which were killed some days after inoculation. It is, therefore, a very natural belief that in those cases in which death was long delayed it was due to tuberculosis. In order to discover whether the diphtheritic exudation acted specifically in the production of tubercle, or whether it merely set up a local inflammation which formed a focus of infection, we experimented by putting under the skin of rabbits small masses of innocuous foreign matters.

SECOND SERIES OF EXPERIMENTS.

Inoculation of foreign bodies subcutaneously.

Number of experiment.	Date of inoculation.	Animal.	Inoculation.	Recovery or death.	Result of autopsy and microscopic examination.	Remarks.
45	Apr. 19	Small rabbit, No. 16.	A piece of wood, fragment of a match, put below the skin in the posterior part of the neck and the wound closed by a suture.	Wound healed rapidly, but subsequently a small lump of cheesy matter was formed. The animal was feverish for several days. In the lapse of two weeks the lump disappeared, and the animal remained well to date, June 8.	.....	The foreign bodies used here have been before the experiments thoroughly washed and cleaned.
46	Apr. 22	Small albino rabbit, No. 17.	Treated similarly.....	Wound healed rapidly, but within a few days a large lump formed; ulceration set in, and cheesy matter protruded from wound; animal became emaciated, feverish. Died May 13.	All organs appeared hyperæmic; liver contains several small abscesses and numerous small nodules, the latter also seen in lung and spleen; lymphatic glands swollen; microscopic examination revealed large collections of tubercle granulations in all organs; the proportion of white blood corpuscle increased; no bacteria in blood.	
47	Apr. 22	Small rabbit, No. 18.	A piece of clean glass put below the skin in the right thigh; wound closed.	Result similar to last, only slower. Died May 20.	Lesions similar to the last, but slighter in degree; hemorrhagic infarctions in lungs very marked.	
48	Apr. 25	Large albino rabbit, No. 19.	A piece of glass put deep below the skin in the right thigh; wound closed.	Wound healed at first, then severe ulceration set in, cheesy matter protruding from wound; the latter increased to four times the original size. Died May 10.	No decided lesions to the naked eye; microscopic examinations showed all organs to be profusely tubercular; hemorrhagic infarctions in the lungs; echinococci cysts in the liver; no bacteria in the organs.	
49	Apr. 25	Albino rabbit, No. 20.	Treated similarly.....	Wound healed after slight suppuration. Animals remain well.		
50	Apr. 25	Small rabbit, No. 21.	Treated similarly with a piece of cork.			
51	Apr. 25	Small rabbit, No. 22.	Treated similarly with a small bunch of clean hair.			
52	May 6	Small rabbit, No. 23.	A piece of wood, fragment of a match, put deep below the skin in the right thigh.	Cheesy lump formed rapidly and protruded from wound. Died May 19.	All organs showed masses of tubercle granulations; no other decided lesion; no bacteria in the organs	
53	May 6	Small rabbit, No. 24.	A piece of wire put below skin in the left thigh.	Cheesy lump formed; the wire ulcerated away. Died May 15.	Hemorrhagic infarctions in lungs; tubercle granulations everywhere, although not very marked; no bacteria in the organs; none in the blood.	

It will be seen that in five out of nine of these experiments tubercle was found after death; this large proportion apparently demonstrates that a simple local inflammation may in the rabbit act as a source of tubercular infection. Now, when in our first experiments rabbits were inoculated with diphtheritic matter, inflammation was almost always induced at the seat of the lesion, with the formation of large lumps containing cheesy matter. These facts being so, it is a fair deduction that the tubercles were secondary to the local inflammatory foci, and were therefore an indirect and not a direct result of the inoculation.

These experiments seem to warrant the deduction that inoculation with materials taken from patients suffering from the endemic mild diphtheria of Philadelphia frequently produces a secondary or indirect tuberculosis in the rabbit, but very rarely, if ever, causes any disease in the rabbits comparable to diphtheria in man, or even any septic disorder; of course the experiments do not prove that malignant cases of diphtheria may not occur in Philadelphia and have different relations to inoculation. Indeed, the groups of experiments omitted from the discussion (Exp. 37 to 41, inclusive) prove that matter taken from malignant cases of endemic diphtheria does produce in rabbits a rapidly fatal disorder.

The method by which Trendelenburg asserts that he succeeded in producing diphtheria in rabbits consists in placing the exudation matter in the trachea. We naturally have suspected that the membrane when placed in the trachea produces simply a trachitis. This suspicion has been strengthened by the observation that acute pseudo-membranous trachitis and angina occur in rabbits. Such an epidemic destroyed, during the winter of 1879, a number of rabbits kept by one of us in a clean but damp place. The rabbits first showed sickness by refusing food; examination then detected swelling of the tonsils with exudation. There was a high fever with increase of the local symptoms until the animals became entirely unable to swallow. Death occurred in from three to seven days, preceded by great difficulty of breathing and profound exhaustion. False membrane was abundant in the mouth and trachea, and on examination showed all the characteristics of diphtheritic exudation.

In October, 1882, we observed a similar disease (spontaneous pseudo-membranous anigina and trachitis), from cause unknown, in white rats. Three out of seven animals died with very marked local lesions and the blood crowded with micrococci. The symptoms and phenomena of the disease were identical with those just described in the rabbit epidemic. The room in which the animals were kept in cages was very cold and draughty, but as well as the cages had been kept very clean and no diphtheritic material had been brought to the laboratory within three months. Six months previous these same rats had been inoculated with diphtheritic material, but had recovered and remained well until the time stated.

In order to determine whether the tracheal inoculation of rabbits with diphtheritic membrane taken from the mild type of the disease will cause pseudo-membranous trachitis, the following experiments were performed:

THIRD SERIES OF EXPERIMENTS.

Inoculation with diphtheritic matter in the trachea.

Number of experiment.	Date of inoculation.	Animal.	Inoculation.	Recovery or death.	Result of autopsy and microscopic examination.	Remarks.
54	May 31.	Large albino rabbit, No. 27. Fresh rabbit.	Inoculated with dried diphtheritic matter, mixed with water, in the trachea, from without.	Recovered and remained well...	.....	Case X.—Of diphtheria, received through Dr. Jaggard, about 24 hours after removal. The membrane dry and hard; apparently in perfect condition.
55	June 1.	Albino rabbit, No. 10. See experiment 10.	Same as last	Died June 6, 5 days after inoculation, in convulsions.	External wound had healed perfectly; some ecchymosis noticeable, and the subcutaneous tissue infiltrated and congested. The wound in the trachea not quite healed; larynx and trachea congested and covered by a delicate true pseudo-membrane, which reaches, near the larynx, a thickness of 1mm. Microscopically, it also appears fully identical with the natural and with the ammonia false membrane, containing micrococci in large number. No bacteria in blood, none in organs. The organs are tubercular, more especially the liver, where large nodules can be seen even by the naked eye. No hyperæmia of organs, as in the ammonia specimen.	Same case.
56	June 14.	Young albino rabbit, No. 37.	Inoculated with dried diphtheritic matter, mixed with water in the trachea.	June 18. Evening. Animals apparently well; take food, &c.; breathing not much interfered with. The same evening killed rabbit 37; No. 38 was found dead June 19.	No lesions; no bacteria in blood, which was very carefully examined.	Case II.—Diphtheritic matter taken by Dr. Richard A. Cleeman from throat of patient untouched; inoculation about 36 hours after membrane was taken; it was dried quickly and was in good condition.
57	June 14.	Young albino rabbit, No. 38.	Same as last		No lesion; blood not examined.	Same as last.

These experiments show that the membrane of endemic diphtheria will produce a pseudo-membranous trachitis, and the question arises, is the pseudo-membrane proof of a specific trachitis, or is any trachitis of sufficient intensity accompanied by the formation of a false membrane? To answer this query the following experiments were undertaken:

FOURTH SERIES OF EXPERIMENTS.

Injection of ammonia into the trachea.

Number of experiment.	Date.	Animal.	Inoculation.	Recovery or death.	Result of autopsy and microscopic examination.	Remarks.
58	May 15, 4 p. m.	Large albino rabbit, No. 11.	Injected three or four drops of aqua ammoniae into the trachea from without, the trachea being laid bare and a small opening cut into it.	Died May 18, 1 p. m., in convulsions, 69 hours after inoculation. During sickness breathing of the animal was extremely affected, very forced, deep, the rabbit opening the mouth widely at each straining effort, and raising the head; did not take food except the last twelve hours before death, when he seemed to feel easier.	Wound in skin and muscles covering the trachea was suppurating; tracheal wound had healed. All organs strongly hyperæmic, and tubercular nodules recognizable by naked eye. On opening the larynx and trachea a well developed pseudo-membrane of 1 to 3mm in thickness was seen, which reached below the bifurcation and into the smaller bronchia. It resembles fully in color, consistency, and easiness of detachment, the natural croupous membrane, and is perfectly identical with the latter upon microscopic examination. Micrococci are abundant, both in spheres and disseminated; none in internal organs except the lungs. Tubercles in the lungs, spleen, lymphatic glands. Hemorrhagic infarction in lungs.	
59	May 15, 4 p. m.	Large albino rabbit, No. 12, about 4 years old.	Treated with ammonia similarly to the foregoing.	Died May 18, 4 p. m., 73 hours after inoculation. Animal seemed not to suffer and took food well; death in convulsions, which lasted about 3 hours.	External wound as well as that of the trachea had perfectly healed. Lesions perfectly similar to those of foregoing rabbit only less intense in degree. Tubercles more scarce; lungs less hyperæmic and less infarcted. No micrococci in blood; pseudo-membrane fully developed and perfectly similar to foregoing. Bacteria and micrococci present in membrane and in lungs, but none in other organs.	
60	May 28, 12 m.	Albino rabbit, No. 7.	Treated with ammonia like foregoing rabbits, experiments 58 and 59.	Died May 30, 11 a. m., 71 hours after operation.	Lesions fully identical with last rabbit, experiment 59.	
61	May 28, 12 m.	Cat, No. 7.....	Same as last experiments, 58, 59, and 60.	Died May 30, 2 p. m., 50 hours after operation.	Lesions and well-developed false membrane similar to those in the last three rabbits, but here absence of tubercles. No micrococci in blood, and none in organs.	

62	May 28, 12 m.	Dog, No. 3.....	Same as last.....	Great difficulty of breathing and inability to swallow followed the operation, but animal was artificially fed with milk, &c.; 12 days after the operation seemed to be recovering; killed June 9th.	Body much emaciated; skin wound healed, while tracheal wound was open yet; slight congestion of tissues around trachea. In many places on the mucous membrane of trachea traces of disappearing false membrane seen; whole trachea covered by thick tenacious mucus containing large quantity of luecocytes, some giant cells, and micrococci in moderate quantity. Spleen highly tubercular, tubercles in liver, lymphatic glands, and some in lungs. No micrococci in blood taken from jugular vein immediately after death.	
63	June 8, 4.30 p. m.	Fresh rabbit, No. 25, about three months old.	Treated with ammonia similarly to foregoing five experiments.	Died June 9, 4 p. m.; no convulsions.	Autopsy made immediately after death. Wound in skin healed; tracheal wound open; the tissues around latter strongly hyperæmic; trachea nearly filled by false membrane. A preparation of menbrane taken five minutes after death showed the usual elements of a natural diphtheritic membrane with great abundance of micrococci; the blood did not contain micrococci. Lungs much congested; its vesicles largely filled with the croupous exudation, blood corpuscles, and micrococci. All other organs normal and not containing micrococci.	
64	June 8, 4.30 p. m.	Rabbit, No. 26.	Same as last	Died immediately from effect of operation, too much ammonia having been given.		

The experiments which are recorded in the last table show that ammonia is able to produce in the cat and dog, as well as in the rabbit, a pseudo-membranous trachitis. Professor Oertel states that the membrane produced by cauterization of the trachea differs from diphtheritic membrane in containing no micrococci. What has led him to such an assertion, we cannot comprehend. In all our studies, when the death occurred very quickly, micrococci were somewhat less abundant in the traumatic membrane than in that taken from the throat of patients, but when the animal survived some days and the micrococci had sufficient time to develop themselves—when, in other words, they were afforded as good opportunity of growth as in the natural disease—they were immensely abundant, in some cases seeming to make up a large part of the bulk of the membrane.

A priori we would expect this result since we have demonstrated that there is no physical difference to be found between the micrococcus of the mouth and the micrococcus of diphtheria. When then, trachitis under the influence of the irritant, is set up and exudation poured out, it seems a necessity that the micrococcus shall rapidly spread in the soil thus provided for it. To see whether organic irritants other than diphtheritic exudations will produce a pseudo-membranous trachitis, the following experiments were performed:

FIFTH SERIES OF EXPERIMENTS.

Inoculation with foreign bodies, pus, &c., in the trachea.

Number of experiment.	Date.	Animal.	Inoculation.	Recovery or death.	Result of autopsy and microscopic examination.	Remarks.
65	May 19.	Large rabbit, No. 14. See experiment 21.	Inoculated in the trachea with slough from a bed sore.	Wound healed rapidly, the animal recovering completely.		
66	June 3	Small rabbit, No. 28. Fresh rabbit.	Inoculated in the trachea and in the thigh muscles with exudation from throat of a scarlet fever patient.	Died June 12	Large cheesy lump on thigh. Some congestion and translucent mucus around tracheal wound which had not healed; no false membrane. No lesions in the organs; not examined for bacteria.	Case X.—From Dr. Fulton, the matter being produce of ulceration of a scarletinal sore throat.
67	June 3.	Small rabbit, No. 29.	Inoculated in the trachea only with the same matter as last.	Died June 10	Skin wound healed; upon dissection a cheesy abscess found below subcutaneous tissue, pressing upon the trachea, and probably having been the cause of death. No other lesions perceptible. No bacteria in blood. None in organs.	
68	June 8.	Small rabbit, No. 30.	Inoculated in trachea with the pseudo-membrane produced by ammonia in rabbit No. 25.	Animal well, June 18		
69	June 8.	Small rabbit, No. 31.	Same as last	Killed June 16	No lesions except congestion of trachea and large cheesy lump between the trachea and skin.	
70	June 8.	Small rabbit, No. 32.	Inoculated in trachea with purulent mucus taken from trachea of dog No. 3, experiment 62.	Died June 11	No lesions in any organ perceptible; tracheal wound not healed. No bacteria in blood.	
71	June 9.	Large rabbit, No. 33.	Inoculated with ichorous pus in trachea.	Animal well		
72	June 10.	Small rabbit, No. 34.	Inoculated with pus in trachea.	Found dead June 11	No lesions except congestion of trachea.	
73	June 10.	Small rabbit, No. 35.	Same as last	Died June 18	Large cheesy lump between skin and trachea. Wound in trachea not healed; on opening the trachea a distinct pseudo-membrane of from 1 to 1½ millimeters in thickness was found, prominently seen only below the tracheal wound, *i. e.*, in the lower half of the trachea and the bifurcation. Microscopically, this membrane was identical with the	

Fifth series of experiments—*Inoculation with foreign bodies, pus, &c., in the trachea*—Continued.

Number of experiment.	Date.	Animal.	Inoculation.	Recovery or death.	Result of autopsy and microscopic examination.	Remarks.
					natural diphtheritic membrane and with those produced by introduction of ammonia, and of diphtheritic matter in the trachea. Cheesy abscess was found in the left lung; many air vesicles of both lungs filled with a croupous exudation containing multitudes of micrococci; prominent miliary tubercle in all organs. No micrococci in the kidneys; blood not examined.	
74	June 10	Small rabbit, No. 36.	Inoculated with ichorous pus in trachea, and also deep in muscle.	Died June 17.....	Large cheesy lump upon trachea the latter much congested. Within the trachea a very distinct pseudo-membrane was developed having the same macroscopic and microscopic character, as that of last experiment. Croupous inflammation of lungs. Other organs normal.	

In looking over the last table, it will be seen that in two of the ten experiments pseudo-membranous trachitis was caused by the introduction of organic matter into the trachea. In both of the cases in which false membrane was produced the injected material was pus; and it will be noticed that only four such experiments were made, so that the proportion of successful result is very large; larger, indeed, than with true diphtheritic exudation in our experiments.

Trendelenburg found that not only ammonia, but also various other chemical irritants are capable of causing the formation of false membrane in the trachea. Many years since it was proven that tincture of cantharides will do the same thing. It would seem, therefore, that in the trachea the formation of a pseudo-membrane is not the result of any peculiar or specific process, but simply of an intense inflammation which may be produced by any irritant of sufficient power. The experiments so far detailed seem to establish the following propositions:

First. That it is difficult to produce in the rabbit a rapid septic disorder with the matter taken from ordinary cases of so-called diphtheria as we see them in Philadelphia.

Second. There are, however, certain malignant cases of diphtheria occurring in Philadelphia, inoculation with membrane from which produces rapid illness and death in the rabbit.

Third. That tracheal inoculations with membrane from endemic diphtheria will sometimes cause a pseudo-membranous trachitis.

Fourth. That both septic animal matter and non-organic irritants placed in the trachea cause pseudo-membranous trachitis which cannot be distinguished from diphtheritic trachitis, the membrane in both cases containing micrococci.

Fifth. The occurrence of a false membrane in the trachea is the result not of the specific character but of the intensity of the inflammation.

Section B.—EPIDEMIC DIPHTHERIA.

After studying the subject of endemic diphtheria, we next made trial of membrane from cases of the epidemic diseases. In the following report the membrane was obtained in experiments 75 to 89, inclusive, at Ludington; in the remainder, at Lakeview.

SIXTH SERIES OF EXPERIMENTS.

Inoculation with epidemic diptheritic matter from Ludington.

[The term "pyæmic abscess," used on several occasions, is meant to express a small collection of pus or cheesy matter made up of *débris*, cells, and micrococci not included in a cyst, with or without evidences of echinococci in them.]

Number of experiment.	Date.	Animal.	Inoculation.	Result.	Autopsy and microscopic examination.	Remarks.
75	June 23.	Rabbit.....	In trachea with matter (from Roche case), material three days old.	Died June 26	*Autopsy one hour after death.*—A huge diphtheritic ulceration occupying whole front of neck. Trachea full of exudation, which extends to the pharynx, tonsils, roof of mouth, and back of tongue; exudation not very tough, full of micrococci; bone-marrow (examined fresh) filled with micrococci. Microscopic examination of organs showed—lung croupous pneumonia; liver and kidneys, cloudy swelling, with hemorrhagic infarctions, and only a few of the arterioles and capillaries blocked up with micrococci masses; spleen normal, and not containing micrococci.	The diphtheritic material was obtained at Ludington, patient died, and the inoculation made in Philadelphia.
76	June 23.	....do	.....do	Died June 27	Two hours before death, blood taken in tubes from the jugular coagulated instantly, and contained an enormous amount of micrococci. *Autopsy immediate.*—At place of wound around trachea, large ulceration filled with exudation, which was composed of leucocytes, fibrin and micrococci; mucous membrane of trachea inflamed; larynx filled with pseudo-membrane. The latter contained abundantly micrococci. Lungs, croupous pneumonia; rest of organs normal, and no micrococcus emboli after very careful examination.	
77	June 23.	....do	.....do	Died June 28	Blood examined after death full of micrococci; a large sloughing diphtheritic ulcer at seat of inoculation, with much exudation composed largely of micrococci, but containing numerous rod-bacteria also; trachea inflamed, containing very little exudation.	

					Double croupous pneumonia; also empyæma. Rest of organs normal. No micrococci could be discovered in any of the organs except in the air-vesicles of lung.
78	June 23.	....do	In vagina with diphtheritic matter.	Negative	
79	June 23.	....do	Diphtheritic material put into mouth and throat without scarification.	.....do	
80	June 23.	....do	Diphtheritic membrane from Smith's case, three days old, placed in pocket of muscle of thigh.	Died July 6.....	*Autopsy immediately after death.*—Blood full of micrococci; a large ulcer, with abundant diphtheritic exudation composed chiefly of micrococci, at seat of inoculation; liver full of small pyæmic abscesses (?). Marrow of bone full of micrococci. Kidney and liver contained some micrococcus embali; rest of organs normal.
81	June 23.	....do	.....do	Died June 26, seventy-one hours after inoculations. Blood from ear examined four hours before death contained micrococci, not very abundantly.	*Autopsy a few minutes after death.*—Blood from jugular vein full of micrococci. At seat of inoculation an almost gangrenous diphtheritic ulcer, surrounded by widespread œdema of subcutaneous cellular tissue; few pyæmic abscesses in liver; lung croupous pneumonia and some purulent pleurisy. Rest of organs normal, and not containing micrococci.
82	June 23.	....do	Diphtheritic membrane four days old (from case of Nelson) placed under skin.	Was sick, with periods of getting better, until July 11, when it died.	*Autopsy some hours after death.*—Blood contained no micrococci; did not coagulate rapidly; in no way perceptibly abnormal. At seat of inoculation a cheesy lump with micrococci. Liver with small pyæmic echinococci cysts filled with granular matter and pus; widespread tuberculosis of internal organs; no micrococci found in bone-marrow and spleen (which were examined).
83	June 23	....do	Same mattter as last put in pharynx by scarification.	Negative	
84	June 23.	....do	Same matter as last put in eye; conjunctiva scarified and eyelids sewed up tightly.	At first considerable local inflammation about eye, which soon subsided; animal seemingly recovered, but was found dead July 16.	Liver with pyæmic abscesses; other organs normal and not containing micrococci.
85	June 23.	....do	A piece of diphtheritic kidney (from Roche case) 3 days old, inserted under skin.	June 30 rabbit very ill; died July 1.	Blood taken from jugular (June 30) by means of sealed tubes clotted at once, was full of micrococci. Autopsy some hours after death. Liver studded with pyæmic abscesses spleen swollen and dark; rest of organs normal, and in none of them could micrococci be discovered.

Inoculation with epidemic diphtheritic matter from Ludington—Continued.

Number of experiment.	Date.	Animal.	Inoculation.	Result.	Autopsy and microscopic examination.	Remarks.
86	June 23.	Rabbit.....	In trachea with matter (from Roche case), material three days old.	Died July 6	*Autopsy immediate.*—Blood full of micrococci; large diphtheritic ulcer at place of inoculation, with very profuse membranous exudation. Liver full of pyæmic abscesses. Organs not examined for micrococci.	
87	June 24.	... do	Hyperdermic injection of about three drops of blood taken four days before from a fatal case of diphtheria in Ludington.	Died July 3.....	No micrococci in blood. No lesions discovered elsewhere.	
88	June 24.	....do	.....do	Negative		
89	June 24.	....do	Injected 4 minims of saliva four days old.	Died July 11.....	*Autopsy.*—No micrococci in blood; no evidence of local lesion; internal organs slightly tubercular.	
90	March 10	Wild rabbit	Left thigh with fresh diphtheritic membrane.	Died March 14.....	Blood from jugular some time after death, loaded with micrococci, which were also abundant in spleen and marrow of bone. Large sloughing ulcer at seat of inoculation.	These inoculations were made at Lakeview, Michigan, immediately from the patient to the animal.
91	March 10	Guinea pig	Left thigh, fresh membrane.	Animal sick a few days but recovered.	.....	Same as last. Temperature taken daily during 10 days, at first varied somewhat but later was constant and never below normal.
92	March 11	Wild rabbit	.....do	Died March 14	Blood of heart full of micrococci. Spleen marrow of bone and kidneys also contain micrococci emboli.	The inoculation same as last.
93	March 11	White rat.....	Both thighs	Recovered.....	.....	Do.
94	March 17	....do	Thigh, dried membrane.....	.....do	.....	Do.

An examination of the records just given shows that the Ludington diphtheritic matter acted as a very deadly poison upon the animals inoculated with it. In three rabbits, portions of the membrane were placed in the trachea with uniformly fatal results (experiments 75, 76, 77). In three rabbits (experiments 80, 81, 82) the membrane placed under the skin or in the muscles acted as promptly as did that inserted in the trachea. The experiments in which the inoculation of membrane had no effect were those in which the poison was placed upon an open surface, where it was liable to be washed off. Such were experiments 78, 79, and 83, where the vagina and mouth were selected. In experiment 84 the membrane was more or less imperfectly retained in position by fastening the eyelid over it, and the result was somewhat doubtful.

In two instances (experiments 85, 86), instead of using membrane we employed minute pieces of kidney for inoculation, and with precisely the same effect, namely, rapid death, with characteristic lesions. Injections of minute quantities of blood and saliva into the veins (experiments 87, 88, 89) gave, however, entirely negative results, with one very doubtful exception.

The experiments made with the Lakeview material were not quite so decisive; out of five inoculations only two proved fatal, but these two were the only ones in which rabbits were used. In two others the animals were rats, which, in various other experiments, we have found to enjoy a singular immunity from being affected by the diphtheritic poison. In Europe it has been found that these animals are very slightly affected by any septic virus; and it would seem as though long-continued residence in sewers and similar places through successive generations has produced a power of resisting these organisms, which naturally are to be found in such localities. It ought further to be noted that the malignancy of the Ludington scourge was far greater than was that of the Lakeview epidemic.

It will be seen that the effects produced were entirely different from those which were obtained previously with Philadelphia matter. The result of the two series of experiments may be summed up in a few words, as in the following proposition: Whilst inoculation of the rabbit with false membrane from endemic or ordinary Philadelphia diphtheria is not usually followed by rapid systemic infection, it is so followed when the cases furnishing the false membrane have been malignant; further, the placing of material from cases of malignant epidemic diphtheria in the trachea or under the skin is usually fatal, the characteristic post mortem lesions consisting in the presence of micrococci in the blood, and sometimes also in the internal organs, along with severe inflammation at the point of injury, where there is produced an exudation resembling diphtheritic membrane and crowded with micrococci.

The question which naturally arises at the present juncture is: Is the affection produced in rabbits the same as diphtheria of man? Any one who has carefully studied the able, though not sufficiently known, pamphlet of Drs. Curtis and Satterthwaite will agree with us in believing that they obtained in some cases results similar to those which were yielded by the Ludington material, but in other instances effects like those produced with Philadelphia diphtheritic material. The portion of their text relating to this matter is so important that we quote it in full.

"In the above-described disease we failed to see anything specifically resembling diphtheria as it occurs in the human subject. The whole story seemed to be one of local-irritant poisoning, which always tended toward the production of an abscess at the site of inoculation, with greater or less concomitant hypodermic, ecchymoses, and serous infiltration of neighboring tissues, according to the degree of virulence of the inoculated poison. According, also, to the severity of the primary lesions, the animals would die or survive the immediate effects, and, according to the secondary history of the mass, infiltrated with leucocytes—according, namely, as the mass was reabsorbed or softened and ulcerated—the animal survived unaffected or slowly wasted away and died of exhaustion. But it might, not without reason, be argued that a rabbit is a widely different animal from a man, and that a disease induced by subcutaneous inoculation might naturally differ in its manifestations from the same occurring idiopathically; hence, that the described affection of rabbits might still be true diphtheria, although wanting in the specific characteristics of that disease as seen in the human subject. To determine this point we proceed to try if effects similar to the foregoing would follow the inoculation of a material resembling diphtheritic membrane in its anatomical and chemical character, but yet not only not diphtheritic, but even incapable of producing any noxious effect under circumstances where diphtheritic membrane often proves highly infectious. Such a material presented itself in the scrapings from the upper surface of a somewhat 'furred' tongue from a healthy person. These scrapings, while obviously not infectious when brought in contact with the pharyngeal mucous membrane, yet contain anatomical elements of similar character and in similar vital condition to those of the diphtheritic membrane. They also swarm with countless bacteria.

"Such scrapings, inodorous when fresh, acquire a peculiarly offensive, fœtid smell even within a few hours. Inoculations with this pulpy material were then made in the usual way, with the following results: Three animals were inoculated with the

matter freshly removed. Of these, one died between the fourth and fifth day with a lump in each thigh in all respects similar to that produced by the diphtheritic inoculation. One died on the fourteenth day, with similar lumps, which had softened and ulcerated; and the third on the twenty-fifth day, with widely extended burrowings of pus and secondary peritonitis. Four other rabbits were inoculated with tongue-scrapings twenty-four hours old and fœtid. Of these the first developed a large lump of the usual character in twenty-four hours. This was then removed entire for study, and the animal survived, with no further lesion. The other three animals were let alone; all developed the usual lesions, and all died on respectively the fifth, nineteenth, and twenty-fifth days. A third series of two rabbits were inoculated with the mixed scrapings of five successive mornings, which were exceedingly offensive to the smell. One died on the sixth day with the usual lumps, but as this was a young animal from a litter some of which died without having been experimented on at all, it is an open question whether or not the death in this case was due to the inoculation. In the other rabbit lumps developed, which ulcerated and discharged foul matter. The animal suffered also in its general health, but ultimately recovered.

These series of experiments thus developed the important fact that certain non-diphtheritic and non-infectious matters will, when inoculated upon the rabbit, produce a disease closely similar to, if not identical with, the disease caused by the inoculation of diphtheritic membrane, and one, also, equally fatal in its effects. But inasmuch as tongue-scrapings are open to the suspicion of containing some peculiar poison derived from the animal body, we determined to experiment with substance which should be free from such objection, while still resembling diphtheritic membrane and tongue-scrapings in the matter of being putrescent and swarming with bacteria. We accordingly chose Chon's fluid which had passed into a state of decomposition. This fluid is simply a solution in distilled water of ammonium tartrate, potassium phosphate, and magnesium sulphate, to which also a little calcium phosphate is added. Upon exposure in a warm place, bacteria appear in this fluid in great numbers, and of all the salts present new compounds are formed of a strongly putrid smell. Inoculations were made with this putrescent material, as follows: Two animals were inoculated in the usual way with the pulpy sediment forming at the bottom of the bottle and obtained by straining the fluid. Both developed the usual purulent lumps, but with little attendant irritation or constitutional disturbance. In both the lumps gradually disappeared by absorption and atrophy, and the animals survived. Four rabbits were inoculated by hypodermic injection with the lower stratum of liquid in another sample of the decomposed fluid. Lumps, as usual, formed at the site of each injection; one animal died on the tenth day; the other survived. Four rabbits were injected hypodermically with the upper stratum of liquid in still another sample. Lumps again appeared at each inoculated spot, but they were small, and all the animals survived.

"The same procedure in four other rabbits with a fourth sample of fluid produced like effects in three of the animals, but in the fourth no lumps discoverable by feeling the spot could be found. Fourteen animals in all were thus inoculated with the putrid Cohn's fluid. Thirteen of them developed lesions identical in appearance with the characteristic lesions from inoculations with diphtheritic membranes and tongue-scrapings, but the collateral effects were not so severe, and only one animal died from the effects of the inoculation. In short, then, putrid Cohn's fluid, inoculated upon the rabbit poisons after the same manner as diphtheritic membrane, though not to the same measure.

"It would seem, therefore, that the disease produced in the rabbit by inoculations of diphtheritic matter is not only not specifically diphtheritic in character, but not even peculiar to the diphtheritic infection, since a disease essentially similar, if it be not pathologically identical, is producible, though in variable intensity, by inoculations of material at once non-diphtheritic and non-infectious to human mucous membrane, and even, it may be, not of animal origin. This fact being apparently established, the important corollary follows, that pathological and pathogenetic conclusions drawn from the effects of diphtheritic inoculations of the rabbit do not, of necessity, apply to the disease, diphtheria, as it appears in the human subject. The many current hypotheses in the premises, therefore, which rest upon such animal experimentation are built upon a quicksand.

"This conclusion being accepted, it was plain that to attempt to elaborate the pathology of the diphtheritic process by study of the rabbit's inoculation disease would be a waste of time, and all thoughts of the same were accordingly abandoned. But though the inoculation disease is not necessarily nor even probably diphtheria, yet it is possible, and indeed probable, that the poisonous element in the diphtheritic membrane, which will produce diphtheritic infection in man, is the same kind of thing as that which produces the inoculation disease in the rabbit. Hence it seemed to us to be a useful research to try to discover the nature of the infecting principle, whether of diphtheritic membrane, tongue-scrapings, or Cohn's fluid, which produces the inoculation disease."

We give this extract in full because it seems to us very important, although we think there is no difficulty in showing the incorrectness of the conclusions reached; indeed, we believe that if the authors of the paragraph had had at their command facts now established, they would have arrived at different conclusions.

The fact that something else besides diphtheritic membrane will produce in the rabbit symptoms similar to those caused by the membrane does not disprove that the disease developed by the diphtheritic inoculation in the rabbit is diphtheria; it may be that putrid tongue-scrapings are capable of acting as a diphtheritic infective matter. In a previous chapter we showed that the micrococci of such tongue-scrapings are not distinguishable from those of diphtheria.

The most important objections to the conclusions drawn by Drs. Curtis and Satterthwaite are not, however, to be found in such reasoning as this. It is certain that in most cases the authors quoted failed to produce anything like diphtheria in the animal, and it is most probable that they never produced with Cohn's fluid anything more than a local inflammation. We have proven that almost any substance placed under the skin of the rabbit is capable of producing death, and that such death is preceded by the formation of cheesy lumps. The membrane of malignant diphtheria kills the rabbit rapidly without producing these lumps. Evidently the tongue-scrapings, &c., used by Curtis and Satterthwaite acted like the membrane from our Philadelphia cases, and did not cause true systemic infection.

On the other hand, if we examine the general constitutional symptoms exhibited by the systematically infected rabbit, we find they are a progressive failure of strength, with fever, such as are seen in the man suffering from diphtheria. If we examine the local symptoms the similarity is even more close; a membrane not to be distinguished by the microscope from that of diphtheria in the trachea, or if the inoculation has been made in the muscle, a local exudation similar in all its elements to this membrane. Drs. Curtis and Satterthwaite seem to rely upon the fact that in inoculated animals membranes do not form, except at the point of inoculation. We do not think this cogent. The cases of diphtheria in which membranes form elsewhere than at the original point of disease are certainly in the minority in man; and according to the best modern pathologists, it is probable that these secondary formations are the result of secondary local infections, and are not the direct result of the constitutional disturbance. Would any one deny that a case of disease in a child was diphtheria because membrane did not form elsewhere than in the throat?

Finally, the post-mortem lesions are not to be distinguished in the rabbit dead of an artificial diphtheritic trachitis and a child dead of a malignant diphtheritic trachitis. As, therefore, the poison is the same in the rabbit and in the man, as the symptoms induced are very similar and the post-mortem results identical, we are forced to believe that the disease produced by the inoculation of the rabbit with malignant diphtheritic membrane is essentially diphtheria.

Again, on page 101, we report some experiments which we have made to determine whether it is possible to pass the disease from rabbit to rabbit, and which show that the animal diphtheria does not differ from human diphtheria in its contagious power.

Further, there can be doubt of the spontaneous occurrence of diphtheritic epidemics among the lower animals. As examples of records of such, we would refer, *inter alia;* to the *Deutsche Zeitschrift f. Thierheilk*, 1877, III, p. 1; also, *Ibid*, 1878, p. 64, for very excellent studies of the affection as it occurs in calves, by Professor Dammann and by F. Blâzekovic; and to the *Marseilles Medical*, xvi, 1879, p. 104, for an account by Dr. W. Nicati of epidemics among pigeons. These and other articles we might cite are in accord with the studies made on rabbits and rats by Dr. Formad and prove that there is a contagious epidemic disease, which affects especially the young of domestic animals, and is exactly similar in its etiology, its clinical phenomena, and its post-mortem lesions to diphtheria in man. It is this disease, which we believe, that we have cased in our rabbits by inoculation. The *Experimentum crucis*, the absolute proof of the identity of human diphtheria with that of the lower animals would be its production by inoculating human belngs with membrane taken from diseased animals. For obvious reasons we have not made any such experiments, but we have found in medical literature several reported cases in which the production of human diphtheria has been ascribed to the animal virus.

The account of the first of these cases we know only as it is quoted by Mr. George Fleming, a veterinary inspector of the British war office (*Veterinary Journal*, 1881, p. 166), who states that a Mr. Macgilloray asserts that the disease (diphtheria) has been very common amongst calves in his practice for fifteen years, and that "isolated cases of sore throat often occur among farm servants, when ugly looking, yellowish spots or deposits are found in various parts of the throat."

Not having been able to obtain a copy of the original journal we are indebted to the New York Medical Record for the statement that Mr. Cole, a veterinary surgeon of Hinckling, in Australia, published in the *Australian Veterinary Journal*, February, 1882, a report on a severe epidemic of diphtheria amongst the lower animals, with 'an account of an epidemic (human) that occurred in the Oakleigh police station,

the disease being, on this occasion, traced to a diseased cow, whose milk had beer used by the inmates of the station."

Mr. George Fleming (*Veterinary Journal*, September, 1881) is inclined to believe that the contagious disease known as "garget," or "infectious mammitis," in cows is a form of diphtheria. In 1879 a very violent outbreak of diphtheria in one of the London districts was officially investigated by Mr. W. H. Power, medical health inspector, who traced it to the milk supply, and came to the conclusion that the epidemic was due to the existence of "garget" among the cows (*Medical Times and Gazette*, January, 1879, p. 67). It is also stated in the journal just quoted, "that during the recent outbreak of diphtheria in the Princess May Home garget was found to be prevalent at the farm supplying milk to the home. We do not, however, attach very much weight to this evidence. It only has force enough to excite attention on the part of medical men having opportunities for observation as to the relations between the infectious mammitis and diphtheria.

Much the most decisive of the reported instances of the transmission of dipththeria from the lower animals to man is that which is detailed by Professor Dammann in the *Deutsche Zeitschrift für Thiermedicin*, 1877, III, p. 1. Professor Dammann's position as director of the Hannover Veterinary School, and the highly scientific careful method of his study and report of the epidemic give great weight and authority to his observations. The outbreak of the epidemic was among calves and the contagiousness of the disease was thoroughly established. At the suggestion of Professor Dammann, the veterinary officer in charge began, April 29, to make local applications to the mouths and throats of affected calves. On May 5, after some premonitory throat symptoms, he became ill, and four days later Professor Dammann found him confined to bed, with a high fever, complaining of very sore throat, great weakness, thirst, and cephalic distress; there was swelling of the cervical and submaxillary glands and slight membranous exudation upon the now highly inflammed tonsils; a day or two later, according to the report of his attending physician, the membrane in the throat became very well developed; subsequently the disease followed the usual course of a severe, but not malignant, diphtheria. The dairy maid, who took the place of the inspector in waiting upon the calves, was similarly but less severely affected. The origin of the outbreak among the calves could not be distinctly established, but it was traced with probability to the child of a coachman, who died of diphtheria at the hostelry before the first calf sickened.

We have, therefore, in the paper of Professor Dammann a clear record by a thoroughly competent observer of an epidemic of diphtheria in calves probably originating in man, and certainly spreading from animal to animal and from animal to man. This, taken with the other similar recorded cases and our own experimental facts, seems to us to establish the identity of human and animal diphtheria.

SEVENTH SERIES OF EXPERIMENTS.

Inoculation with diphtheritic products from animal to animal.

Number of experiment.	Date.	Animal	Inoculation.	Result.	Remarks, autopsy, and microscopic examination.
95	June 26	Rabbit	Piece of heart clot from rabbit No. 7 put under skin.	Died July 4	*Autopsy some hours after death.*—External wound healed, with small cheesy lump; no micrococci in blood; organs all tubercular.
96	June 27	do	Inoculated with false membrane from rabbit No. 2.	Died July 13	*Autopsy some hours after death.*—External wound healed, with very small cheesy lumps; organs tubercular; no micrococci in blood.
97	June 28	do	Put piece of membrane in jugular from rabbit No. 3.	Negative	
98	July 6	do	False membrane from rabbit No. 1 put in trachea.	Died July 9	*Autopsy two hours after death.*—Blood full of micrococci, also bone marrow; trachea with a moderate amount of pseudo-membrane; lung croupous pneumonia and purulent empyæmia; spleen small, atrophied; liver with pyæmic abscesses; rest of organs normal and not containing micrococci.
99	July 6	do		Died July 14	*Autopsy.*—Large diphtheritic ulcer at point of inoculation, the slough containing all kinds of bacteria, but micrococci predominating; no micrococci in blood, spleen, or marrow of bone; no pyæmic abscesses in liver.
100	July 10, 9 a. m.	Albino rabbit	Hypodermic injection in back of blood of rabbit of experiment No. —, just dead of diphtheritic inoculation.	Found dying in the evening.	Profuse congestion at seat of lesion from the hypodermic injection. Blood full of micrococci in all forms (the blood used to kill this rabbit apparently was free from micrococci). Organs not examined.
101	July 10	do	do	Died July 11	Profuse local lesion; purulent cellulitis. Blood the same as in last experiment. Marrow of bone show micrococci and fine micrococci emboli in kidney and liver.
102	July 10	do	Same as last, blood from just dead rabbit.	Died July 16	Purulent local cellulitis. Micrococci in blood and in white blood corpuscles in moderate quantity. Organs not examined.
103	July 16	Small albino rabbit	do	No result	
104	July 16	Small gray rabbit	do	do	
105	July 16	Large black and white rabbit.	do	do	
106	March 14	Guinea pig	Inoculation in skin of neck with membrane from a pig died of diphtheria.	Died March 18	Sloughing wound. Blood full of micrococci. Organs not examined.
107	March 15	White rat	Inoculated in neck, with membrane from a pig as last.	Died March 20	Sloughing wound with abundant false membrane. Micrococci abundant in blood, which also contained a few bacilli. Marrow of bone and spleen crowded with micrococci.

These experiments certainly demonstrate that not only the local exudation but also the blood of animals dead of diphtheritic infection is capable of inducing similar infection in previously healthy animals. Those made with material originally from Ludington (experiments 95 to 99 inclusive) indicate that passing through the system of the rabbit lessens the activity of the poison, since in about half the cases tuberculosis and not acute systemic infection was produced. The remaining experiments of the series do not, however, accord with this, the animal virus showing itself to be very deadly.

The question now presents itself squarely before us, *What are the relations of the micrococci which exists in diphtheria to the disease? Are they the poison of the disease, or, in other words, are they capable of producing it?* The absolute final answer to this question can only be made by producing disease in the animal by micrococci isolated from the remainder of the diphtheritic poison. The difficulties to be overcome in such an attempt are very apparent. Three classes or lines of experiments at this juncture present themselves:

First. The study of the influence of filtration upon the poison as contained in the diphtheritic membrane.

Second. The study of the action of the isolated micrococci as they are to be obtained from urine.

Third. The study of the action of micrococci raised in culture chambers entirely away from the original poison.

Fourth. The effect of boiling upon the infectious properties of diphtheritic membrane.

In regard to the first of these methods, it is plain that the attempt must be to filter as completely as possible all solid particles from membrane, rubbed up with water; and also that if it be found that the filtrate is innocuous and the matter remaining on the filter still poisonous, the only logical deduction is that the poison is particulate and not soluble. It is impossible by this method alone to progress further and prove that the micrococci, and not the bits of fibrin, white blood cells, &c., are the *materies morbi*.

Drs. Satterthwaite and Curtis made an infusion of diphtheritic membrane and filtered it through a double paper filter. The filtrate, which was clear and odorless, was injected hypodermically into three rabbits. All survived, two without even any local lesion, but the third with a large lump of the usual character, containing living bacteria upon one side. None of the animals seemed to suffer in general health. At the same time the unfiltered infusion was injected into three other rabbits, and all of them died before the end of the third day with severe lesions.

It is evident that these experiments, as far as they go, indicate that filtration takes out the poisonous properties of diphtheritic material, since the formation of the lump in the side of one of the rabbits of the first series was due simply to local irritation. Want of material prevented Doctors Satterthwaite and Curtis from carrying this potionr of their research any further.

We have made a number of filtration experiments, using always the vacuum filter, sometimes forcing the liquid through porous clay cylinders such as are employed in certain galvanic batteries, sometimes drawing it through three or four thicknesses of filtering paper. The liquid for filtration was obtained by triturating the membrane with water. In some cases the membrane was allowed to macerate in the water for a few hours before the filtration.

The experiments are given in two tables, the first containing those in which the liquid filtrate was injected, the other those in which the solid matters left upon the filter were employed. A "syringeful" equals about half a fluid drachm.

EIGHTH SERIES OF EXPERIMENTS.

Experiments with liquid filtrate from diphtheritic matter.

Number of experiment.	Date.	Animal.	Inoculation.	Result.	Autopsy and microscopic examination.	Remarks.
108	May 31	Rabbit, moderate size...	Injected one drachm of the liquid hypodermically.	Died June 9.....	No local lesion except a little ecchymosis at place of injection beneath skin. No micrococci in blood, neither before nor after death. Organs normal. Death could not be accounted for.	Inoculated with a hypodermic syringe with liquid which had been filtered once through a clay filter; on microscopic examination no micrococci were found in the liquid but only a few bacterium termo. Fatal case (Döngés child). Matter fresh.
109	May 31	Albino rabbit.....	.....do	Died June 14.....	No micrococci in blood. Autopsy revealed right-sided heart clot but no lesion, except some decided congestion of all the internal organs. No cellulitis at place of injection.	Do.
110	May 31	Small black rabbit.....	.....do	Remained well.....	.....	Do.
111	June 24	Small Albino rabbit.....	Injected subcutaneously 10 minims of the diphtheritic liquid (clay filtrate).	No result	.....	Inoculated with liquid obtained by clay filtration of water in which membrane from (Dr. Montgomery) fatal case has been soaking 30 hours. Microscopic examination showed absence of micrococci; a very few rod bacteria to be seen.
112	June 24	Large gray buck rabbit.	Injected three syringefuls of the same liquid.	.....do	.....	Do.
113	June 24	Large black and white rabbit.	Injected two syringefuls of the same liquid filtrate.	Died July 10.....	Extensive cellulitis at seat of injection (back of neck). The œdematous liquid below the skin contained some micrococci. Blood also showed a few micrococci. A few white blood corpuscles contained them. Organs appeared normal; no microscopic examination was made of them.	

114	June 24	Small gray rabbit.....	Hypodermic injection of one syringeful of liquid clay filtrate.	No result	.....	Do.
115	June 24	Black French hare	.....do	.... do	.....	Do.
116	June 24	Wooly rabbit.....	Same as last, only dose increased to three syringefuls injected into different parts of the body.	.....do	.....	Do.
117	June 24	Albino middle-sized rabbit.	Same as last, two syringefuls.	Died July 1.....	Some cellulitis at seat of injection. Matter scraped off from seat of lesion showed some micrococci and some bacilli. Blood normal. Organs normal, except lungs highly congested.	Do.
118	June 24	Large gray and white rabbit.	.... do	No result	....	Do.
119-24	July 8 10 a. m.	Six young French hares of one litter.	Each of these six hares was given hypodermically a syringeful of the clear clay filtrate above mentioned into muscle of right and left thigh.	Two of them found dying July 9, one died in the evening; two more were found dead July 10; one survived and remained well.	None of these five hares showed any micrococci in the blood or in the white blood corpuscles. There were some bacteria in the œdematous liquid at the inflamed cellular tissues at seat of the injection. Organs of one of those hares were saved and examined, but did not show micrococci or any lesions except some congestion. One of the hares showed peritonitis.	Inoculated with filtered liquid from membrane which was 36 hours old, dating from death, and was excessively stinking. Filtration through clay. No micrococci could be found in liquid, but only a few bacterium termo.
125	July 8	Gray rabbit	Two syringefuls of same liquid hypodermically.	Remained well.....	....	Do.
126	July 8	Large Albino rabbit....	Hypodermically three syringefuls.	.....do	.....	Do.
127	July 8	Gray and white rabbit..	Same as last	Died July 25.....	Had several cheesy lumps below skin, furrowed by slight cellulitis; no œdema; no micrococci in blood, none in organs.	Do.

NINTH SERIES OF EXPERIMENTS.

Inoculation with solid residue from diphtheritic matter.

Number of experiment.	Date.	Animal.	Inoculation.	Result.	Autopsy and microscopic examination.	Remarks.
128	May 31	Rabbit of moderate size.	In right thigh	Died June 3	Micrococci in blood from ear one day before death; temperature at this time was 97° Fahr. (four degrees below normal). Blood crowded with micrococci and white blood corpuscles showed loculation and micrococci. Micrococci found also in marrow of bone and spleen. Sections of kidney and liver were made and stained with aniline, and numerous, micrococci emboli were discovered. The latter were better demonstrable in acid glycerine preparation.	Inoculated with solid matter (Dönge's child) left on clay filter.
129	May 31	Adult rabbit	In right thigh	Died June 4	Micrococci found in blood from ear on June 2, during life. After death blood showed also numerous micrococci, free and in zooglœa masses, and loculated white blood corpuscles. Organs showed micrococci emboli.	Do.
130	May 31	Small rabbit	do	do	Micrococci in blood; loculation of white blood corpuscles very marked. Organs showed some micrococci emboli.	Do.
131	July 9	Brown and yellow, large rabbit.	Inoculated in neck below skin and into trachea.	Died July 12	Diphtheritic pseudo-membranous trachitis and left-sided croupous pneumonia. Blood full of micrococci; also in white blood corpuscles, which are loculated. Kidney examined after hardening shows in sections numerous micrococci emboli. Rest of organs was not examined.	Solid matter which had been thoroughly rubbed up with water, and after this had been filtered off had been repeatedly washed on the filter with water. (Case of Dr. Kollock.)
132	July 9	Middle-sized gray rabbit.	Inoculated below skin of neck only.	Recovered	Wound healed perfectly	Do.
133	July 9	Middle sized gray and white rabbit.	Same as last, in left groin.	Found dying July 14	Wound sloughing; pseudo-membranous lymphatic glands greatly swollen. Blood contains some micrococci in white blood corpuscles, but apparently now free. Spleen and kidney examined, but very few micrococci found. There were also some bacilli in the kidneys.	Do.
134	July 9	Large yellow rabbit	do	Recovered	Wound healed	Do.
135	July 9	Small French hare	Inoculated in muscle of back.	Remained well	do	Do.

In looking over these records of experiments it will be seen that there were 20 inoculations with filtered liquid and eight with solid materials, and that of the first series ten, or 50 per cent., died; whilst of the second series, five, or 60 per cent. of the inoculations resulted fatally. This at first sight indicates that filtration has but little effect upon diphtheritic matter, but a close study reveals a very different state of the case. In no case when a liquid filtrate was employed were there decided local evidences of diphtheritic inflammation at the seat of inoculation, and in no instance were there unmistakable signs of general systemic infection, such as abundant micrococci in the blood and internal organs; indeed in only a single instance were a few micrococci found in the blood, and even in this case the internal organs escaped. Several of the deaths were produced by secondary tuberculosis. Take these out and take out also the very fatal experiments made on July 8 (Exps. 119–124) and the deaths are reduced to a minimum. The cause of five out of six rabbits inoculated on July 8 (Exps. 119–124) dying so quickly is not clear, but points to the existence of some poison in the fluid used other than that of diphtheria. It must be remembered that the weather was intensely hot, that the membrane had been obtained in quantity at a post-mortem examination, and was in a state of partial decomposition, and that a large quantity of concentrated infusion was employed. Under these circumstances it is very probable that some alkaloidal or other soluble chemical poison was present in the stinking mass and rendered the infusion a deadly poison. However this may have been, it is plain the animal did not offer either the symptoms or lesions of diphtheria and did not die of diphtheria. Of course filtration does not remove absolutely all of the microscopic solid particles; a few will escape, and occasionally a filtrate may act feebly in producing the disease.

Further, when micrococci are so infrequent in a liquid microscopic examination may readily fail to detect them. When it is remembered that in no single case out of 20 experiments were distinct diphtheritic symptoms produced, although in one or two experiments a few micrococci developed at the point of injection, it seems to us that results more striking than we have obtained could not be expected. Control experiments show that in all instances the matter used was active; the water was brought in contact thoroughly with it and the amount injected into the animals was enormous. Moreover, in the control experiments it was proven that washing with water does not remove the poison from the solid mass. We must therefore consider it established that the poison of diphtheria is solid and particulate, and is not soluble in water.

As already stated in some detail in our biblographical review, Dr. Letzerich has found that if the clear urine of a patient suffering from diphtheria be run through paper filters, and these filters afterward washed so as to remove all possible soluble contaminating matter, and then dried, a paper is procured full of micrococci, but containing no other of the urinary elements except it be minute traces of epithelium, &c. The prepared paper Dr. Letzerich found to be as poisonous as is diphtheritic membrane.

In repeating these experiments of Dr. Letzerich we did not wash the filters before inoculation, so that our experiments may not seem to be as conclusive as they otherwise would be. When, however, it is remembered that the poison has been shown to be insoluble, solid, and particulate, and that it has been proven that water is unable to remove from diphtheritic material its noxious properties, the failure to wash the filter loses all importance.

TENTH SERIES OF EXPERIMENTS.

With filter taken from urine.

Number of experiment.	Date.	Animal.	Inoculation.	Result.	Autopsy and microscopical examinations.
136	June 23	Rabbit....	Vagina without erosion.	Negative.	
137	June 23	....do	Filter paper in mouth; scarified and tied up mouth.	Died June 25, about 36 hours after inoculation.	Autopsy some hours after death. Blood taken by tubes from the jugular vein full of micrococci in large numbers, both free and in zoogloea masses. No membrane in m. m. mouth, larynx, or trachea. Lungs pale and collapsed. Liver full of small abscesses. Other organs normal. No micrococcus emboli found in organs.
138	June 22	....do	Filter paper as last.	Negative.	
139	June 23	....do	Filter paper put under skin of thigh.	Died during night of June 25.	Autopsy some hours after death. Blood full of micrococci; white blood corpuscles full of micrococci; numerous balls of them in blood and zoogloea masses also. At seat of inoculation a sloughing ulcer covered with diphtheritic exudation with much œdema around it. Liver with pyæmic abscesses. Other organs normal and containing micrococcus emboli.

The experiments just reported show that the filter paper loaded with micrococci from the urine is even more poisonous than is the membrane itself. It will be remembered that in no case did we succeed in inoculating with the membrane placed in the mouth of the rabbit, yet one out of two experiments with the filter paper was successful. No vaginal inoculation with any kind of diphtheritic matter has in our hands succeeded, so that the failure of experiment 136 goes for nothing. In the only remaining trial the paper with the micrococcci was placed under the thigh, and death occurred in less than three days, the blood being crowded with micrococci and the internal organs markedly affected.

In order to determine whether ordinary urine contains any solid material poisonous to rabbits, we inoculated four of these animals with filter paper through which had been passed large quantities of normal urine. In one instance the paper was placed in the trachea through an artificial opening; in the other rabbits the pellets were put in the muscles. None of the rabbits were sickened. The tracheal wound healed rapidly.

The force of the urine-filter experiments seems to us very great. The amount of other solid particles in the urine was so small that it is very improbable that the particulate poisons which caused the diphtheria was other than the micrococci. As the experiments have been performed by three observers (Letzerich and ourselves), their correctness can scarcely be challenged. They seem to us to prove that the micrococci are either the poison, and by their growth enter the tissues and destroy the blood corpuscles, or else that they by the processes of growth in the body produce a poison which is the true *materies morbi*, or else that they are saturated with the poison and act as carriers of it.

Our next series of experiments were made to determine whether it is or is not possible to produce diphtheria with the artificially cultured micrococci. In them a piece of blotting-paper, which had been moistened with the culture fluid containing growing micrococci, was placed in the tissues or trachea of the rabbit. The experiments are as follows; those from experiment 140 to experiment 160, inclusive, were made in Philadelphia from cultivations of material obtained in Ludington; whilst experiment 161 to experiment 180, inclusive, were made at Lakeview from cultivations of fresh material:

ELEVENTH SERIES OF EXPERIMENTS.

Culture inoculations.

Number of experiment.	Date.	Animal.	Inoculation.	Result.	Autopsy and microscopical examinations.
140	August 8	Rabbit	First generation of micrococci from Ludington diphtheric matter in trachea.	Died August 9	Autopsy some hours after death. No micrococci in blood; no local or other lesions.
141	August 8	do	As last	Died August 23	Autopsy: Trachea healed; no micrococci in blood.
142	August 8	do	do	Died August 26	Blood examined just before death; no micrococci in it. Autopsy, pneumonia.
143	August 9	do	Fifth generation Ludington matter in thigh.	Died August 24	Autopsy some hours after death. No lesion in solid tissues; no micrococci in blood.
144	August 9	do	As last	Negative	
145	August 9	do	do	Died August 27	Autopsy: General tuberculosis.
146	August 9	do	do	Negative	
147	August 9	do	do	do	
148	August 9	do	do	do	
149	August 15	do	Inoculated in the muscle of the thigh with third generation, Ludington matter.	do	
150	August 15	do	do	do	
151	August 15	do	do	Died August 23	Autopsy some hours after death. No micrococci in blood.
152	August 15	do	do	Negative	
153	August 15	do	do	Died August 29	Autopsy some hours after death. General tuberculosis.
154	August 15	do	do	Negative	
155	August 15	do	Inoculated in trachea with third generation of Ludington matter.	do	
156	August 15	do	do	do	
157	August 15	do	do	Died August 17	Autopsy some hours after death. No micrococci in blood. Croupous pneumonia. No pseudo-membrane in trachea, but a simple catarrhal trachitis.
158	August 15	do	do	Died August 28	Autopsy: No micrococci in blood; croupous pneumonia; other organs normal.
159	August 15	do		Died August 28	Autopsy: No micrococci in blood; general tuberculosis.
160	July 13	do	Inoculated with the second generation of the diphtheritic matter in the thigh.	Died July 21	Blood examined just before death; contained micrococci in considerable quantity, infesting white blood corpuscles and also some free. Autopsy immediately. Filter-paper still in position surrounded by an inflamed area, but no pseudo-membrane. Liver does not contain abscesses; marrow of bone containing micrococci both in the cells and free; other organs congested, but no micrococci emboli.

An examination of the records of these experiments shows that there is only one in which it can be affirmed that diphtheria was produced by the inoculation with cultured micrococci. This was the experiment 160 made with the second generation. All the experiments made with the third and fourth generations failed, or at least yielded very doubtful results, in that after death no distinctive evidence could be found of the existence of false membrane at the place of inoculation or of micrococci in the blood. In experiment 160 the result was somewhat decisive. There is, however, a special reason why all the experiments, save the 160th should have proved unsuccessful. The diphtheritic matter was two and more weeks old at the time of inoculation, as our first studies were naturally directed to the morphology of the micrococci under culture. It will be shown later on in our memoir that the fungus must have lost some of its vitality; moreover, we allowed the individual generations of the cultures to grow longer than we should have done. Moved by these considerations and aided by the generosity of the National Board of Health, Dr. Formad went to Lakeview, Mich., and there made a series of cultures and inoculations. They are as follows:

ELEVENTH SERIES OF EXPERIMENTS—Continued.

Inoculation with cultures from Lakeview diphtheritic matter.

[Made in Ann Arbor, Mich.—Dr. Herdman's laboratory.]

Number of experiment.	Date.	Animal.	Inoculation and matter used.	Result.	Autopsy and microscopical examinations.	Remarks.
161	Mar. 19	Guinea pig No. 4	Hypodermic injection of culture liquid (10 minims) containing a pure crop of micrococci, 4th generation culture, 4 days' standing.	Died March 24.....	Micrococci in blood of heart. Extensive cellulitis with œdema at seat of inoculation. Organs showed micrococci emboli.	
162	Mar. 19	Guinea pig No. 5.....	Hypodermic injection of the same, into peritoneal cavity.	Died March 21.....	Micrococci in blood. Profuse purulent peritonitis. No examination of organs.	
163	Mar. 19	Middle-sized black rabbit.	Treated similar to Guinea pig No. 4.	.....	On immediate autopsy no micrococci could be found in blood. Profuse cellulitis at seat of inoculation and oedematous liquid containing large amount of micrococci.	
164	Mar. 11	Wild rabbit	Second generation of micrococci from case 2. Lakeview diphtheric matter.	Died March 16	Micrococci found everywhere in blood and organs. Sloughing wound.	Temperature fell below normal 12 hours before death.
165	Mar. 12	Wild rabbit	Third generation of micrococci from case 2. Lakeview diphtheric matter.	Died March 20	Cheesy lump at the seat of wound, with micrococci in tissue around it. Wound healed. No micrococci in blood, some in marrow.	Therm. observations daily, March 13 to 20: 102° F., 100°, 101°, 102¾°, 100°, 98°, 97°.
166	Mar. 10	Guinea pig	Second generation of micrococci from case 2, Lakeview diphtheric matter.	Found dead March 20	Micrococci in blood and everywhere in organs.	Therm. observations from March 11 till March 19, daily: 103½° F., 103°, 99°, 100°, 100°, 99½°, 97°, 99°, 96½° (on the day before death.) The animal sickened on the second day after inoculation.
167	Mar. 11	White rat	Second generation of micrococci from case 2, Lakeview diphtheric matter.	No result	Some local inflamation, which subsided soon	

Eleventh series of experiments—*Inoculation with cultures from Lakeview diphtheritic matter*—Continued.

Number of experiment.	Date.	Animal.	Inoculation and matter used.	Result.	Autopsy and microscopical examinations.	Remarks.
168	Mar. 11	White rat	Second generation of micrococci from case 2, Lakeview diphtheric matter.	Recovered	March 15, fine false membrane in the thigh	
169	Mar. 12	White rat	First generation of micrococci from case 2, Lakeview diphtheric matter.	Died March 18	At seat of inoculation much serous exudation with micrococci. Blood loaded with micrococci and bacilli.	Therm. observations from March 12 (evening) till March 18, daily: 102° F., 101°, 103½°, 104°, 93, 94½. Animal all the time ill, with much diarrhea last days.
170	Mar. 14	Guinea pig	Inoculation second generation, fresh-made and kept outside of incubator in cold place 4 hours.	Died March 18	Wound healed. No lesions or micrococci to be found anywhere.	
171–3	Mar. 15	Three white rats	Third generation	Negative		
174–5	Mar. 16	Two white rats	Liquid filtered away from second generation; it showed very few if any micrococci.	Negative		
176–8	Mar. 17	Three white rats	Inoculation with fourth generation.	Negative		
179	Mar. 17	White rabbit	Fluid filtered from second generation.	Died March 21	No local lesions. No micrococci in the blood, or other apparent cause for death.	
180	Mar. 17	Wild rabbit	Fourth generation hypodermically.	Negative.		

Many of these experiments were made with rats, because it was very difficult to obtain the requisite supply of rabbits at Lakeview during Dr. Formad's visit to the place. At that time we did not know, what we have since abundantly proven, that rats are infected with diphtheritic poison only with difficulty. Dwelling in drains, sewers, outhouses, and similar positions for generations, the domestic rat seems to have acquired a power of resisting all septic organisms, as has been shown not only by our experience, but also by that of various European workers. In one of the experiments (169), however, the first generation of culture did produce a fatal diphtheritic infection, and in another case (experiment 168) the second generation caused a local inflammation, with formation of false membrane. Of experiments upon animals other than rats there are five in which diphtheritic symptoms and lesions were produced. The generations employed were from the first to the fourth. It will be seen, therefore, that in seven cases we have produced unmistakable results with cultivated micrococci, and that in several other instances have had evidences of infection, but that we have never been successful beyond the fourth generation.

It is true that in a number of cases we have failed to produce the disease; but this only rarely when the original stock was fresh. All of the experiments which were made at Lakeview are reported.

It has been established by the labors of Pasteur (address before the International Medical Congress, 1881) and others that fungal organisms are capable of losing their noxious powers under altered conditions of cultivation. For reasons which shall be detailed hereafter, we believe that this is eminently true of the micrococcus now under consideration. Failure, therefore, to produce diphtheria with the third generation of cultivated micrococci from old stock is no evidence that the micrococci is not the *materies morbi* of diphtheria. The explanation of such failure is probably to be found in the tendency of the micrococci to relapse into its inert stage or state under the more or less defective conditions of artificial cultivation. Then, again, we are convinced that quantity is a very important factor in the action of the diphtheritic micrococci; that not only must the plant be in an active state, but that also it must be in sufficient amount at the place of inoculation. The cultivated plant exists in very minute amount as compared with the natural plant, and in our culture inoculations the amount of the plant inserted into the animal was very small as compared with the mass inserted where membrane or kidney was employed. Whatever may be the cause of failure in some cases, it is certain that we have produced diphtheritic septicæmia with cultures up to the fifth generation.

It is evident that we have proven that the poison of diphtheria is solid and particulate; that it is found in the urine when the only solid particles present are micrococci, and it is producible by micrococci which have been grown entirely away from any animal organism. It seems to us established that micrococci cause diphtheria by their power of forcing their way into the tissues, and by their own excessive vitality overcoming the vitality of those tissues, changing their nutritive processes, destroying them, and finally getting into the blood itself, destroying its white corpuscles and obstructing the circulation; during this infective process it is very probable that the micrococci may by assisted by chemical principles, which are developed during the growth of the fungi, and which act as depressing poisons to the human system, although they are not the poison of diphtheria, and if existing by themselves in the blood would not cause diphtheria, but a general vital depression.

If the poison of diphtheria be, as we believe has been shown, micrococci, boiling the membrane for a length of time should deprive it of its noxious properties. The application of a temperature of 212° Fahr. may be endured for a short time by these lower organisms, and it is even possible that prolonged boiling may be withstood by some of them; but our present knowledge indicates that this is not the case. We have made a number of experiments as to the effect of high temperatures upon the toxic properties of diphtheritic membranes, and append an analysis of them:

SERIES TWELFTH.

Inoculations with boiled diphtheritic membrane, made at Lakeview.

Number of experiment.	Date.	Animal.	Inoculation.	Result.	Autopsy and microscopical examinations.	Observations of temperature, and remarks.
181	Mar. 11	Wild rabbit	In thigh, with membrane boiled for 5 minutes.	Found dead March 15	Micrococci in blood. Organs not examined.	
182	Mar. 11	do	do	Animal found dead March 19.	Unhealthy ulcer showed micrococci. Micrococci in blood and marrow.	Temperature of body 10 hours after inoculation: 102° Fahr.; March 12, 103½°; March 13, 101°.
183	Mar. 11	do	Second generation; boiled 5 minutes.	Found dead March 18	Sloughing unhealthy wound. Micrococci in blood in moderate quantity. No other lesions.	Thermometrical observations Mar. 12–18: 102° Fahr., 103°, 102½°, 100½°, 101°, 99°, 96½°.
184	Mar. 10	Guinea pig	Right thigh, with membrane boiled for 5 minutes.	Died March 17	False membrane in unhealed wound. A few micrococci in blood; more in marrow and spleen.	Thermometrical observations Mar. 10–16: 106° Fahr., 99°, 98°, 99°, 98°, 96°.
185	Mar. 11	White rat	Boiled membrane in thigh. The boiling was done in a test tube for 5 minutes.	Died March 19	Wound sloughing and gangrenous. Micrococci, and also motionless and movable bacilli, in blood. Kidney contained micrococci emboli.	Thermometrical observations daily March 11–18: 101° Fahr., 103°, 102½°, 104°, 99°, 100°, 97°, 98°.
186	Mar. 15	do	Boiled membrane from diphtheritic pig, boiled 15 minutes.	Recovered		This membrane culture chamber failed to show multiplication of micrococci.
187	Mar. 15	do	Boiled membrane for 15 minutes; inoculated in neck.	do		
188	Mar. 15	do	do	do		
189	Mar. 14	Guinea pig	Boiled pig membrane 15 minutes	Found dead March 19	Sloughing wound. No micrococci anywhere.	
190	Mar. 16	White rat	Membrane boiled 15 minutes	Negative		
191	Mar. 15	do	do	do		
192	Mar. 19	Large white and gray rabbit, mother of the preceding litter of young rabbits.	Hypodermic injection of the liquid filtrate from matter used in last experiment.	do		There was an abscess; this discharged and healed perfectly.
193	Mar. 19	Large Albino buck rabbit.	Fresh diphtheritic membrane from fatal case of diphtheria, boiled at a temperature of 107° for two hours in some water; after filtration through fourth filter paper, the residue, about 1 dram, was put below skin of neck.	do		

SERIES TWELFTH—Continued.

Inoculations with boiled diphtheritic membrane, made at Lakeview.

Number of experiment.	Date.	Animal.	Inoculation.	Result.	Autopsy and microscopical examinations.	Remarks.
194	Mar. 19	Guinea pig No. 1..	Fresh diphtheritic membrane from fatal case of diphtheria, boiled at a temperature of 107° for two hours in some water; after filtration through fourth filter paper, the residue, about 1 drum, was put below skin of neck.	Died March 22.....	.....	Death occurred while being shipped in a narrow cage from Ann Arbor, Mich., to Philadelphia. Autopsy could not be made.
195	Mar. 19	Guinea pig No. 2..	.....do	Negative	.....	Both animals alive yet.
196	Mar. 19	Guinea pig No. 3..	Treated similar as rabbit of experment.	.....do	.....	
197	Mar. 19	Young rabbit No. 1.	Hypodermic injection of ten minims of second generation of cultivated diphtheritic micrococci (of five days' standing, good pure crop), boiled for two hours at T. 107°.	.....do	.....	
198	Mar. 19	Young rabbit No. 2.	.....do	.....do	.....	
199	Mar. 19	Young rabbit No. 3.	The same with third generation (boiled).	.....do	.....	

SERIES THIRTEENTH.

Inoculations with boiled diphtheritic material from Philadelphia cases.

Number of experiment.	Date.	Animal.	Inoculation and material used.	Result.	Autopsy and microscopical examination.	Remarks.
200	July 8, 5 p. m...	Large albino rabbit...	Inoculated with boiled fresh diphtheritic matter into muscle of neck (large quantity of material employed).	Died July 20....	Wound covered with pseudo-membrane, sloughing. Blood contained moderate quantity of micrococci in white blood corpuscles and free. Organs contained beautiful micrococci emboli, but also some few bacilli.	The diphtheritic matter was obtained 36 hours post mortem (Blockley Hosp.; Dr. Kollock); boiled for 20 minutes in some water over spirit-lamp.
201	July 8	.....do	.....do	Recovered	.....	Wound healed perfectly.
202	July 8	Middle-sized albino rabbit.	Inoculated with same matter in left thigh.	....do	.....	Wound very soon healed.
203	July 8	Maltese rabbit.....	.....do	Died July 22....	Wound was cheesy, and sloughing extending into joint. Few micrococci in blood, some in marrow. Other organs not examined.	
204	July 8	.....do	Inoculated in muscle of back..	Negative	.....	Lesion was not observed.

On looking over this table it will be seen, first, that there were five experiments in which the diphtheritic material was boiled only five minutes, and that in each case death with abundant evidences of diphtheritic infection occurred, although in one instance the diphtheritic matter was a culture fluid. Second. That there were eighteen experiments in which the boiling was kept up for fifteen minutes or longer, and that in only one case (experiment 200) was there clear diphtheritic infection. In experiment 203 a few micrococci were found in the blood, but the wound was in a condition of cheesy rather than diphtheritic inflammation, and the case must be considered as one of very doubtful specific infection; in all these experiments (200 to 204, inclusive) a very large amount of material was put under the skin, and it was but natural to expect local trouble, even if the diphtheritic poison was destroyed. In the only other experiment open to any doubt (experiment 189) no micrococci could be found at the seat of the inoculation or in the blood, so that the death was, without reasonable doubt, the result of an unhealthy sloughing, but not specific, wound produced by the operation.

The first set of experiments of the present series show that exposure to a temperature of 212° F. for a few minutes is not sufficient to destroy the noxious powers of the diphtheritic poison, but the last set certainly indicate that the infective powers of the membrane do not survive a longer exposure, and the whole series warrants the conclusion that prolonged boiling destroys the infective properties of the diphtheritic membrane at the same time as it kills (see experiments 186, 187, 188) the micrococci. Of course this conclusion corroborates the theory that the micrococci are the *materies morbi*.

In all our experiments with diphtheria we have noticed that unless local symptoms are developed at the seat of the injection no systemic infection follows. The evidence we have brought forward certainly shows that usually diphtheria is primarily a local disease; we have made a few experiments in order to determine whether diphtheria is ever primarily constitutional, *i. e.*, whether the micrococci are capable of entering the blood at once before they effect a local lodgment and of sustaining themselves in the vital fluid so as to cause the disease.

SERIES FOURTEENTH.

Injection of fresh diphtheritic matter into the jugular vein.

Number of experiment.	Date.	Animal.	Inoculation.	Result.	Autopsy and microscopical examination.	Remarks.
205	July 8 ..	Large black and white rabbit.	Injected directly into jugular vein 10 grains of fresh diphtheritic matter rubbed with some salt solution.	Negative	....	The vein was exposed by dissection and subsequently ligated. Examining the matter before injection it showed a multitude of micrococci, granular *débris*, leucocytes, and degenerated epithelial cells. Wound healed and animal recovered.
206	July 8 ..	Large gray and white rabbit.	..... do	Found dying July 9, morning.	Wound beginning to slough. Blood showed moderate quantity of micrococci free and in the white blood corpuscles. Microscopic examination of organs showed micrococci emboli everywhere.	
207	July 8 ..	Large black and white rabbit.	.....do	Died July 14.....	Wound not healed and covered by enormous pseudo-membrane. Blood showed many loculated cells filled with micrococci, also zoogloea masses. Intense croupous pneumonia of both lower lobes. Marrow of bones and kidneys showed a few micrococci masses.	
208	July 8 ..	Middle sized brown rabbit.	.....do	Negative	.....	Wound healed.

These experiments are too few to be decisive, but, when taken with previous evidence, they are sufficient to show that the diphtheritic micrococcus is specially prone to produce a local before a general disease. In two of the experiments (experiments 205, 208) an enormous mass of active micrococci were injected directly into the blood and so swept into the torrent of the circulation that they could not make a local lodgment, and in both these experiments the animals survived. On the other hand, in two other experiments (experiments 206, 207), the same membrane having been used as in the other two experiments, the micrococci did get a local hold, and the animal died.

If these results be contrasted with those obtained with the poison of anthrax, in which the blood is the chief seat of the disease, the primarily local character of diphtheria becomes very apparent. We do not say that it is impossible in diphtheria for the blood to be primarily affected, but it seems to us very evident that such cases are rare and that the habit of the affection is to begin as a local disease. The practical import of this conclusion must be at once apparent to every one.

A very interesting accidental or natural, so to speak, experiment has come under our notice, which strongly confirms this view of the primarily local character of diphtheria. One of the families visited by Dr. Formad in Michigan lived in the woods in a rather isolated place. Nevertheless, four of the children suffered with the disease. The slops from the sick room were thrown out with the kitchen refuse and eaten by the pigs. One of these latter speedily sickened and in a few days died. An autopsy was made by Dr. Formad and the stomach with the lower end of the œsophagus was found in a state of intense inflammation and covered with a dense, very thick, false membrane, presenting all the macro- and microscopic appearance of a true diphtheritic exudation. It was simply loaded with micrococci. Moreover, the blood of the pig was full of micrococci both in zoogloœa masses and in the white cells, and micrococci emboli were found in the kidneys, spleen, &c. Further, in a number of experiments we produced fatal diphtheria in rabbits and other animals by inoculating them with material taken from the pig.

The pig undoubtedly died of gastric diphtheria, in which a local affection of the stomach produced a general systemic disorder.

A very important practical point to which we have paid some attention is as to whether diphtheritic matter loses its infective power. When we were working with the Ludington material our time was for some months so closely occupied with the more immediate necessities of our research that we were not able to make any experiments as to when the membrane begins to lose its activity; but the following experiments show that after a time the membrane, even if kept perfectly dry, does lose its contagious powers.

SERIES FIFTEENTH.

Inoculated with two months' old dry Ludington diphtheritic material.

Number of experiment.	Date.	Animal.	Inoculation.	Result.	Remarks, autopsy, and microscopical examinations.
	1882.				
209	Aug. 29	Rabbit....	Dried diphtheritic membrane in muscle.	Negative..	
210	Aug. 29	... do	.....do	... do	
211	Aug. 29	....do	.....do	... do	
212	Aug. 29	....do	.....do	... do	
213	Aug. 29	....do	..... do	....do	All these rabbits had been previously inoculated with culture plants.
214	Aug. 29	....do	.....do	... do	
215	Aug. 29	....do	.....do	... do	
216	Aug. 29	....do	.....do	....do	
217	Aug. 29	....do	.....do	... do	
218	Aug. 29	... do	..... do	... do	
219	Sept. 11	... do	Dried membrane in muscles.	... do	
220	Sept. 11	... do	Dried kidney in muscles....	... do	
221	Sept. 13	... do	Dried membrane in trachea		
222	Sept. 13	... do	.....do	Died October 1.	*Autopsy some hours after death.*—No micrococci in blood, no severe trachitis or any signs of pseudo-membrane; severe double pneumonia. Rest of organs normal and no micrococci found.
223	Sept. 13	....do	.....do	Negative..	The last four experiments were primary inoculations, *i. e.*, rabbits had not been previously used.
224	Sept. 13	....do	.....do	Do.	

These experiments do not seem to need comment. The diphtheritic matter employed had originally possessed the most virulent properties. But in sixteen inoculations, four of them into the trachea, not once did it produce any signs of disease. On placing the micrococci in the culture chambers the reason of this was obvious—they had lost their power of rapid growth, and were as sluggish as plants taken from a furred tongue—a further evidence of the close connection between the power of growth of the micrococci and the infectious properties of the membrane containing them.

We have made a number of experiments with Lakeview membrane, which indicate that the loss of infective power is, at least, under some circumstances, very rapid. The dried membrane was only one or two weeks old. In original infective power we believe it did not compare with the Ludington membrane, which we found when recently dried to be very active.

SERIES FIFTEENTH—Continued.

Inoculation with dried Lakeview diphtheritic matter.

Number of experiment.	Date.	Animal.	Inoculation.	Result.	Remarks, autopsy and microscopical examination.
	1882.				
225	March 16	Large black rabbit.	Inserted into muscle of left buttock dried diphtheritic membrane.	Negative..	
226	March 16	Large mouse colored rabbit.	Inserted into trachea dried diphtheritic membrane.	Do.	
227	March 16	... do	.....do	....do	Breathing very labored and stridulous for two days, but ultimately recovered.
228	March 16	Large white rabbit.	.... do	Do.	
229	March 16	Large pepper and salt rabbit.	.....do	Do.	
230	March 25	Large Albino rabbit.	Inserted into muscle of left thigh dried diphtheritic membrane.	....do	Cheesy lumps formed.
231	March 25	... do	..... do	....do	Wound was sloughing for a while, then healed.

Before considering finally the nature of diphtheria, it seems proper to recapitulate the facts which have been demonstrated in this memoir.

Micrococci are an essential part of the diphtheritic process, being always found locally at the seat of inflammation, and, when blood poisoning develops, also in the blood, attacking and destroying the white blood corpuscles, forming emboli in the kidney, spleen, and other organs.

It is possible to produce in animals by inoculation with membrane, pieces of internal organs, urine, blood, &c., taken from persons suffering from diphtheria, a disease which offers during life the symptoms of diphtheria in man, and after death presents similar lesions, and which is also contagious—capable of reproducing itself; which we therefore consider to be diphtheria. Further, epidemics of diphtheria occur amongst the lower animals, and recorded cases by competent observers show that the diseased animals can produce diphtheria in man.

The poison of diphtheria is solid and particulate.

Washing the solid poison does not remove its toxic properties, and the filtered washings do not cause diphtheria.

The micrococci isolated in the urine or obtained by culture away from the body are capable of producing a systemic diphtheritic infection in the lower animals, if the local lesions develop of sufficient intensity.

Endemic and epidemic diphtheria are similar in their nature, but usually epidemic diphtheria is much more contagious than endemic, although endemic cases do occur possessing intense malignancy and contagious power.

The micrococci of diphtheria do not differ, so far as observed, from the micrococci of furred tongue, &c., except in their tendency to grow in culture fluids.

The micrococci of furred tongue or ordinary sore throat have a less tendency to grow under culture than have the micrococci of endemic non-malignant diphtheria.

The micrococci of endemic or non-malignant diphtheria have a much less tendency to grow under culture than have the micrococci of malignant diphtheria.

The rapidity of growth of the micrococci is in direct proportion to the malignancy of the case yielding them, and its contagiousness.

On exposure to the air diphtheritic membrane of the most virulent type loses its contagious power, and the micrococci *pari passu* lose their power of growing in culture fluids.

Under successive generations of artificial culture the diphtheritic micrococci lose their growth-activity, and also their power of infecting the rabbit.

It has not been experimentally directly proven, but it is a necessary inference from the two facts just stated, that under certain favoring circumstances the sluggish micrococcus puts on growth-activity, and, in all probability, poisonous properties.

Every grade of case can be found in man from an ordinary sore throat, through simple pseudo-membranous angina and trachitis, up to malignant diphtheria.

A case may begin as of sthenic "pseudo-membranous croup" and end as one of adynamic "diphtheria" with blood poisoning; and in cases of this character not infrequently no exposure to contagion is discoverable, and there is clinically every reason to believe that the blood poison has been developed within the body of the patient.

The theory of the disease which we would deduce from these facts is that the micrococcus, which causes the diphtheria, is not a specific organism different from that common to healthy and inflamed throats, but is an active state of that organism; that certain circumstances outside of the human body are capable of throwing this common micrococcus into the condition of active growth and engendering an epidemic of diphtheria. When diphtheria is thus epidemic, the micrococci light upon a throat and, if the throat have little resisting power, as in the child, inflame it or increase a catarrh already existing into a violent inflammation, and also rapidly enter the blood and cause systemic poisoning.

There is a divergence in opinion between the authors of this memoir in regard to the question whether the diphtheritic poison is capable of originating the local inflamation or must find an existing wound or catarrh as a nidus. Dr. Formad believes that the micrococcus can only obtain lodgment and produce the primary local sore when there is a lesion of the parts or an already excited inflamation. According to this view an attack of diphtheria must always be preceded by a simple catarrhal sore throat. Dr. Formad's opinion is founded upon the fact that in no case did we succeed in causing a diphtheritic inflamation by placing the diphtheritic false membrane upon an uninjured mucous membrane of the rabbit.

It is proper to state that our experiments were not made with any intention of testing this point; that those applicable to the question in which a malignant false membrane was used were only very few; and to the thought of Dr. H. C. Wood they only show that the mucous membrane of the rabbit has great resistive powers, and is not as easily inflamed as is the mucous membrane of the child's throat. In none of the experiments was the poison placed in the trachea, where the membrane is delicate and where the micrococci would be little apt to be washed away by secretion. Dr. Wood believes that this experimental negative evidence is of no avail against positive clinical evidence. He considers it absolutely certain that diphtheria is contagious, and if, as we have demonstrated, the micrococcus is the *materies morbi*, clinical facts prove that it is capable of inflaming a delicate throat in which no abrasion exists. Moreover, Dr. Wood believes that there is positive experimental evidence in favor of the view he holds. The case of the pig with gastric diphtheria is in point, since it is altogether improbable that there was any lesion of the stomach before the ingestion of the membrane. The animal was a healthy pig, showing no signs of accident or illness. Then, again, if we have had in regard to this point in a few experiments negative results, other experimenters have obtained an abundance of positive results. Letzerich in especial has succeeded in producing diphtheritic inflamation by placing false membrane upon uninjured mucous membranes, and Professor Dammann has succeeded in epidemic animal diphtheria in causing the disease by placing the membrane in the nostrils of calves. Dr. Wood believes, therefore, that the negative results we have obtained are due to imperfect experimentation; are opposed to positive results obtained by other observers, which positive results are in accord with an immense mass of clinical evidence, showing that the immediate cause of diphtheria is capable of infecting the system, not only by finding entrance through a wound, but also by inflaming a delicate mucous membrane having peculiar susceptibilities and low resistive power, and then, through the abundance of the exudate, causing necrosis of the superficial parts of this membrane and forcing its way through the thus opened lymph spaces in the manner previously explained.

Although the diphthertic attack produced by contagion is caused in this way by a micrococcus from without, diphtheria may also be autogenetic. Thus, a catarrh

in a weakly subject may, in the beginning, be simply an inflammation from cold, but the ordinary micrococci in the throat or mouth, favored by the special conditions, *i. e.*, by the lowered resistive power of the diseased tissue, by the abundance of exudate, and by the increased local warmth, may gradually change from the dormant to the active state and by and by act upon the throat, and at last force their way into the system and a self-generated diphtheria be formed out of a "cold."

CHAPTER V.

ON THE RELATION OF DIPHTHERIA AND ITS MICROCOCCUS TO OTHER DISEASES.

The studies which we have made with the view of determining the relation of diphtheria and its micrococcus to other diseases have not been as complete as desirable, or as definite in their results. Nevertheless they do throw light upon the subject, and we offer them without reserve. They have been in two directions, *i. e.*, clinical examinations and experimental trials.

Examination of blood in measles, Philadelphia Hospital, spring, 1882.

Number of case.	Place.	Sex.	Age.	Day of disease.	Micrococci in blood.	Condition of blood.	Character of disease and termination.	Remarks.
1	Blockley Hospital, Philadelphia.	Male.....	2 years....	Third day.....	Micrococci in large quantity, free and zooglæa masses and infesting the white blood corpuscles.	White blood corpuscles in great excess and many of them loculated.	The eruption had disappeared, but child became daily worse.	The micrococci found in these cases were in every respect similar to those found in diphtheria, the same size and the same behavior to reagents and dyes.
				Ninth day	Micrococci increased in quantity.	The same.....	Child died in the evening of the same day.	Blood taken post-mortem from jugular vein showed multitudes of micrococci. No autopsy made.
2	....do	Female ...	2½ years...	Sixth day.....	Micrococci in blood free and in the cells.	White blood corpuscles in excess, mostly loculated and filled with micrococci. Red blood corpuscles do not form rouleaux.	Bad case; eruption most prominent in throat and mucous membrane; in general not much on skin.	
				Eighth day, three hours before death.	Same as last, only more.	The same.....	Died.....	Post-mortem, the micrococci appeared to constitute a part of the bulk of the heart's clot, which was very large and ante-mortem; organs, however, did not contain micrococci.
3		Male.....	2½ years...	Second day	No micrococci	Normal	High fever.....	Although this case was a typical one of measles, there was decided sore throat covered by pseudo-membrane.
				Fourth day	Blood full of micrococci, free and zooglæa masses and infecting some white blood corpuscles, but none are loculated.	White blood corpuscles increased in number. Red blood corpuscles fail to form rouleaux. Fibrin precipitating freely under the glass.	Worse.	
				Sixth day.....	Micrococci diminished in quantity and not affecting white blood corpuscles.	Same as before.....	Patient better.	
				Eighth day	Diminished in quantity.	White blood corpuscles and fibrin diminished.	Better.	
				Tenth day.....	Still less, but present yet.	Diminishing	.....do	Throat got well.

				Thirteenth day	Some few micrococci seen, free and in short chains.	Blood nearly normal	Doing well.	
				Eighteenth day	No micrococci	Normal	Recovered.	
4	Blockley Hospital, Philadelphia.	Female	8 months	Fifth day	Micrococci both free in blood and affecting profusely the white blood corpuscles, which are mostly loculated.	White blood corpuscles and fibrin in excess.	Bad case (died)	Blood was examined only once. Child died on the ninth day. Large heart clot full of micrococci.
5	do	Male	4 years	Third day	A few micrococci seen in blood.	Normal	Apparently bad case.	
				Sixth day.	Micrococci increased in quantity, but none were noted to affect the white blood corpuscles.	Some increase in quantity of white blood corpuscles. No loculated cells. Red blood corpuscles form good rouleaux.	Child very low	Ulceration in mouth morbid in this case.
				Tenth day	Very few micrococci	White blood corpuscles diminished.	Doing well	Reported to have made good recovery.
6	do	Male	3 years	Eighth day	Some micrococci, but only free and in zoogloea masses, none in white blood corpuscles.	Increase in white blood corpuscles and of fibrin.	Typical case.	
				Twelfth day	Micrococci disappeared.	Normal	Recovered.	

Twenty-three cases more of measles were examined; some once, others a number of times; but in none of these were any micrococci or any loculated cells discovered, although frequently staining and high magnifying lenses ($\frac{1}{12}$ Zeiss) were resorted to. All these cases are also known to have recovered perfectly. Eight cases of Rötheln (German measles) were also examined, but no micrococci found.

Examination of blood in erysipelas.

[Record of cases examined personally during the spring and summer of 1882.]

Number of case.	Place.	Sex.	Age.	Day of disease and when examined.	Quantity of micrococci.	Condition of white blood corpuscles and of blood in general.	Character and termination of disease.	Remarks.
1	Blockley Hospital, Philadelphia.	Male.....	40	Fifth day	Micrococci in large quantity, free and zooglœa masses, but none in white blood corpuscles.	White blood corpuscles decidedly increased in number. Blood highly coagulable.	Affection of leg. Great swelling and redness. Patient not suffering much.	
				Seventh day ...	Micrococci affect white blood corpuscles, some of latter being loculated.	The same	Patient doing well.	
				Ninth day	Very few micrococci, only free and in chains, none in the cells.	Blood nearly normal	Doing well.	Patient recovered.
2	....do	.... do	.....	Second day	No micrococci in blood taken from finger, micrococci in abundant quantity in some bloody serum taken directly from affected part; they were free and in zooglœa masses, and infesting some pus corpuscles.	Blood normal	Affection on face.....	
				Fourth day	No micrococci	The same.....	.....	The local lesion was rather slight.
				Seventh day	.....do	.....do	.....	Patient recovered.
3	....do	.... do	60	Sixth day	Enormous quantity of micrococci free, in zooglœa masses, and in the cells.	Nearly all white blood corpuscles loculated and infested by highly movable masses of micrococci.	Affection on scalp and face; patient shows pyæmic symptoms.	
				Seventh day....	Micrococci diminished in quantity.	White blood corpuscles increased in quantity, but only few affected.	Patient better.	
				Ninth day	Micrococci only few, free and in chains; none in white blood corpuscles.	Blood nearly normal	Doing well.....	There were numerous abscesses in scalp; but patient made ultimately good recovery.
4	....do	.... do	40	Fifth day	Micrococci abundant free and in zooglœa masses.	White blood corpuscles in excess, but none loculated.	Affection of leg; quite extensive.	No more examinations in this case were made, but patient is known to have recovered.

5	...do	Female ...	33	Fourth day	Some few micrococci, free, and also some white blood corpuscles affected.	Blood shows excess of white blood corpuscles and in highly coagulable state.	Facial erysipelas, bad case.	
				Eighth day ...	No micrococci	The same.....	Improving; discharged as cured on the 15th day.	Recovered.
6	....do	.... do	25	Second day	No micrococci in blood. Some blood serum taken from the local lesion contained micrococci in abundance.	Blood normal	Severe case of facial erysipelas (left side).	Local lesion very extensive; the ear, eye, and the whole affected side of face is greatly swollen and discolored.
				Third day	.....	.....do		
				Fifth day	Micrococci, free and in zoogloœa masses, in blood, also numerous loculated cells containing micrococci.	White blood corpuscles enormously increased in quantity, and many are loculated.	Condition of patient worse. Delirium and other cerebral symptoms quite prominent.	The micrococci found here, as well as those of other cases of erysipelas, appeared perfectly identical with those occurring in diphtheria, in regard to size, shape, and behavior to aniline colors.
				Sixth day	Nearly all white blood corpuscles infested by micrococci.	Blood highly coagulable. Red blood corpuscles do not form rouleaux.	Condition worse.	
				Seventh day....	Not examined	.....	Died	Blood examined 12 hours; after death (from heart) showed enormous quantity of micrococci. Blood from finger showed only a few. Autopsy revealed also meningitis.
7	....do	Male.....	50	Fourth day	No micrococci	Increase in white blood corpuscles.	Facial affection.	
				Seventh day	Some micrococci in zoogloœa masses in blood.	A few of the white blood corpuscles infested by micrococci.	Patient doing well.....	Recovered.
8	....do	.... do	42	Sixth day	Micrococci in blood, none in white blood corpuscles.	White blood corpuscles in excess.	Facial affection; doing well.	do.
9	....do	.... do	32	Second day	No micrococci	Normal	Affection of foot.	
				Fifth day	Micrococci in moderate quantity in blood, none in white blood corpuscles.	.....do	Doing well.	do.
10	....do	.... do	18	Fourth day	No micrococci	.....do	Affection of foot.	
11	....do	.... do	26	Third day	Micrococci in moderate quantity.	White blood corpuscles in excess.	Facial affection; severe case.	
				Sixth day	Micrococci in abundance, but none in cells.	The same	Worse.	
				Eighth day	Micrococci less	.....do	Doing well.....	do.

Examination of blood in erysipelas—Continued.

Number of case.	Place.	Sex.	Age.	Day of disease and when examined.	Quantity of micrococci.	Condition of white blood corpuscles and of blood in general.	Character and termination of disease.	Remarks.
12	Blockley Hospital, Philadelphia.	Female ...	29	Third day	No micrococci	White blood corpuscles in excess.	Affection of leg; light case.	
	.....	.....	.....	Seventh day	No micrococci in blood of finger, but large amount of micrococci in serum taken from part affected.	White blood corpuscles not affected.		

Ten cases more of erysipelas were examined for micrococci; each case only once, three with positive and seven with negative results. All the latter were either very light cases, or else in an early stage of disease. The cases were lost sight of and termination unknown.

A study of the tables just given will show that there was examined for micrococci the blood of twenty-two cases of *erysipelas*. In thirteen of these the organisms were found in the blood, whilst in the other nine there were none. Of *measles* twenty-nine cases were studied; in six only were micrococci detected, whilst in eight cases of *Roetheln* or *German measles* there were no organisms. We have also investigated four cases of malignant fatal *scarlet fever*, in all of which we found the blood a few hours before death loaded with micrococci, both free, attacking the white corpuscles, and in zooglæa masses, and in one of which micrococci emboli were abundant in the kidneys. We have also studied four cases of "*puerperal fever*," probably *septic metritis*, in all of which micrococci existed in the blood before death.

It is very certain therefore that micrococci are not peculiar to diphtheria. The question which immediately presents itself at this juncture is, are the micrococci of these various diseases identical among themselves and with the plant that we have found to have etiological connection with diphtheria, or does each disease have its own specific micrococcus?

As already stated, it is very certain that there are no physical differences to be detected even with the highest powers of the microscope; nor is there any difference in the growth of the organisms, either in the blood of the living body or in the culture liquids of artificial propagation.

We have also tried staining with all chemical reagents that we have been able to procure; and the various micrococci have responded in no way different from one another. Of course, not being able to detect differences either physical or chemical, does not prove identity, since, as it is asserted that the specific character of these low organisms are chemical rather than physical, we cannot be certain that in the future some investigator will not discover a method which will determine differences not now to be perceived. All we can say, therefore, is that at present *no physical or chemical differences can be detected between the micrococci of various septic disorders.*

In order to discover, if possible, physiological distinctions, we have made a large number of experiments to determine whether the micrococci taken from erysipelatous affections produce in animals phenomena parallel to those caused by micrococcus diphtheriticus. These experiments are as follows:

Inoculation with fresh erysipelatous pus.

[From case of an iodiopathic erysipelas of face of old man with diffuse purulent cellulitis.]

Number of experiment.	Date.	Animal.	Seat of inoculation.	Result.	Autopsy and microscopical examinations and remarks.
232	May 23	Middle-sized black rabbit.	Inoculation in muscle of thigh	Died June 1	Wound sloughing. Blood contains abundance of micrococci, affecting mainly the white blood corpuscles. Organs congested and contain micrococci.
233	May 23	Middle-sized yellow rabbit.	do	Died June 6	Blood full of micrococci and many loculated cells filled with micrococci.
234	May 23	Brown rabbit	do	Recovered	Wound soon healed.
235	May 23	Middle-sized albino rabbit.	do	Died next day	No micrococci found.
236	May 23	Large albino rabbit	do	Found dying May 27.	Some micrococci found as well as loculated white blood corpuscles.
237	May 23	do	do	Recovered	Wound healed.
238	May 23	Middle-sized albino rabbit.	do	do	Do.
239	June 6	Large albino rabbit	Inoculated in groin	Died June 12	Wound covered with large pseudo-membrane. Glands greatly swollen; cellulitis and large clouds of micrococci in surrounding tissues. Peritonitis. Organs contain micrococci, and nearly all white blood corpuscles are loculated and contain micrococci.
240	June 6	do	do	Died June 17	Wound as in last experiment, but contained only few micrococci. Organs not examined.
241	June 6	do	do	Recovered	Wound healed, although lymphatic glands remained swollen.
242	June 6	Large brown and yellow buck rabbit.	do	do	Wound healed, with encapsulated cheesy lump.

Inoculation from a case of diffuse cellulitis, or phlegmonous erysipelas of the leg.

Number of experiment.	Date.	Animal.	Seat of inoculation.	Result.	Autopsy microscopical examination, and remarks.
243	June 6, morning	Large steel-gray rabbit.	Inoculated in muscle of back	Died June 6	Wound sloughing; great cellulitis around wound; micrococci in blood; white blood corpuscles largely affected; croupous pneumonia; micrococci in liver, spleen, kidney, and marrow of bones.
244	June 6	do	do	Died June 13	Wound covered with large pseudo-membrane; blood loaded with micrococci; most of the white blood corpuscles were loculated and filled with micrococci.
245	June 6	Large brown rabbit	do	Recovered	Wound had healed perfectly on fourth day; micrococci were present, but disappeared.
246	June 6	Large wooly white rabbit.	do	do	Same as last.
247	June 6	Albino rabbit	do	do	Do.

Inoculation with fresh erysipelatous pus from a case of severe erysipelas of face and scalp, with formation of pus in cellular tissues of face and scalp.

[Blood contained micrococci.]

Number of experiment.	Date.	Animal.	Seat of inoculation.	Result.	Autopsy, microscopical examinations, and remarks.
248	July 26	Gray rabbit	Inoculations under skin behind left ear	Found dying July 28, evening.	July 27. Temperature 104½° Fahr., with sudden fall below normal in the morning before death. Great swelling and redness spreading over corresponding side of neck and head, resembling the human erysipelas. Tissues around place of injury were all found crowded with micrococci; blood considerably so. Organs all congested. Kidneys contained some few micrococci emboli.
249	July 26	Albino rabbit	do	Died July 31	Similar to last experiment, swelling of neck and scalp still more prominent. Micrococci present.
250	July 26	Gray and white rabbit	do	Recovered	Both had swelling of cellular tissues around wound, but when latter healed on fourth day it subsided. Micrococci, which were seen in the blood at that time, also disappeared gradually.
251	July 26	Gray rabbit	do	do	
252	July 26	Large brown buck rabbit	Inoculated in lower part of ear	Died July 28, evening.	Temperature on the 27th rose to 107.4°, and fell to 99° Fahr. six hours before death. Ear greatly swollen and inflammation spreading over face. Tissues around place of inoculation crowded with micrococci, also the blood. No micrococci could be found in organs.
253	July 26	Brown wooly rabbit	do	Recovered	Some swelling of the ear, which subsided on third day. Micrococci could not be found in blood.
254	July 26	Albino rabbit	do	do	Temperature rose to 106° Fahr., but soon subsided. At no time could any micrococci be discovered in the blood, which was examined a number of times.
255	July 26	Large albino rabbit	do	do	Wounds healed on second day, swelling subsided, and animals remained well.
256	July 26	do	do	do	
257	July 26	Gray and white rabbit	do	do	No inflammatory changes could be noticed at all.
258	July 26	Large albino rabbit	Inoculation in muscles of neck	Died July 31	Neck greatly swollen. Wound sloughing. Blood full of micrococci, affecting also the white blood corpuscles. Right-sided croupous pneumonia.
259	July 26	Albino rabbit	do	Recovered	Wound had healed perfectly. No micrococci found.
260	July 26	do	do	do	Same as last.
261	July 26	do	do	Died July 31	Blood show considerable quantity of micrococci in all forms of grouping. Organs not examined.
262 263 264	July 26	Albino rabbits	do	Recovered	Wounds had perfectly healed.

Inoculation with bloody pus from unhealthy diffuse cellulitis of calf.

Number of experiment.	Date.	Animal.	Seat of inoculation.	Result.	Autopsy, microscopical examinations, and remarks.
265	July 11	Large black and white rabbit.	Erysipelatous pus in muscle of thigh	Died July 14	Wound sloughing and covered with false membranes. Moderate quantity of micrococci in blood; organs did not show any.
266	July 11	Albino rabbit	do	Died July 13	Same as last.
267	July 11	do	do	do	Temperature rose to 105° Fahr. on July 12, and fell below normal on the day of death (20 hours). Blood full of micrococci. Organs congested, but did not show micrococci.
268	July 11	do	do	Died July 15	Same result.
269	July 11	Gray rabbit	do	Died July 18	Wound not healed. Blood full of micrococci.
270	July 11	do	do	Recovered	Particular attention was paid to the wounds, and they were found perfectly healed.
271	July 11	Albino rabbit	do	do	
272	July 11	Large albino rabbit	do	Died July 19, evening.	Micrococci could not be discovered until the fourth day after inoculation, when they began to affect the white blood corpuscles. Wound did not heal; was slightly sloughing. Temperature at first rose, on second day to 104°, and fell to 99° Fahr. on the day of death. (Average normal temperature of rabbits is 101½° Fahr.) Organs deeply congested, particularly kidneys, and the latter full of micrococci emboli.
273	July 11	do	do	Died July 14	On the 12th the temperature had suddenly risen to 106° Fahr., and fell before death. Blood was loaded with micrococci, which did not appear to affect the white blood corpuscles.
274	July 11	Gray rabbit	do	do	Animal, when died, showed temperature 104½° Fahr. No micrococci could be discovered in blood, and none in organs. Wound was healing rapidly. Death could not be accounted for.
275	July 11	Large albino rabbit	do	Died July 13	Microscopic examination of the pus before used showed it to be made up of leucocytes, red blood corpuscles, granule cells, fibrin, and micrococci in enormous quantity. Blood examined post-mortem; full of micrococci, affecting also white blood corpuscles. Organs not examined.
276	July 11	Large brown rabbit	do	Recovered	Wound in all these instances had healed perfectly within ten days, and when reopened showed a small cheesy lump, and surrounded by dense inflammatory tissue. Micrococci were observed at different periods, but not affecting the white blood corpuscles.
277	July 11	Small brown rabbit	do	do	
278	July 11	Large wooly rabbit	do	do	
279	July 11	Albino rabbit	do	Died July 18, evening.	Micrococci were found during life from third day on. Later examination from fifth day on showed white blood corpuscles affected. Wound did not heal; was sloughing. Organs not sound; they all were deeply congested.
280	July 11	Large black and white rabbit.	do	Died July 19	

There are reported in the series of tables just given the results of thirty-three inoculations made with pus of a most unhealthy character. In twenty-one instances death, preceded by the presence of micrococci in the blood, resulted; and in several instances the animal was very sick, but eventually recovered, the blood during the period of illness containing organisms which disappeared as the symptoms abated.

It is perfectly evident, therefore, that the unhealthy pus of erysipelatous cellulitis not only contains a micrococcus which exactly resembles that present in diphtheria, but also produces, when introduced into an animal, results, both local and general, similar to those caused by the inoculation of diphtheritic membrane.

A point which is worthy of attention is that when pus was employed from idiopathic erysipelas of the face the local effects resembled erysipelatous inflammation, rather than diphtheritic, a diffuse swelling being caused, and a general slough rather than a false membrane. This difference, however, is hardly constant.

We have also made several inoculations with material taken from a case of what might be termed hospital gangrene, as follows:

Inoculation with gangrenous matter.

Number of experiment.	Date.	Animal.	Inoculation.	Result.	Remarks.
281	Aug. 28	Rabbit.....	Inoculated in trachea with matter taken from a case of spreading gangrene, which destroyed the foot of a man in the Philadelphia hospital; it was full of micrococci.	Died Sept. 2, after suffering 2 days with severe trachitis.	Blood full of micrococci indistinguishable from those of diphtheria; inflammation of trachea with false membrane; liver full of collections of micrococci; other organs not examined microscopically.
282	Aug. 28	....do	As the last	Rabbit was ill, but recovered.	

These experiments seem to show that this so-called spreading or hospital gangrene is capable of producing in the rabbit symptoms and lesions similar to those caused by diphtheritic exudation.

In order to probe the matter more deeply we have made experiments with reinoculations.

Inoculations with slough from local pseudo-membrane produced in rabbit which died from inoculation with erysipelatous pus.

Number of experiment.	Date.	Animal.	Inoculation.	Result.	Autopsy, microscopical examinations, and remarks.
	1882.				
283	June 2	Large rabbit..	Inoculation in side of neck; slough from another rabbit, Exp. 232.	Was in dying condition when killed, May 5.	Pseudo-membrane in wound. Blood examined just before death showed a few zooglaea masses of micrococci. marrow of bones contained micrococci in moderate quantity.
284	June 2	Albino rabbit.	In left hind leg, same as last.	Died May 5 ...	No micrococci in blood; otherwise same as last.
285	June 2	Large rabbit..	Inoculation in back...	Found dying, and was killed May 5.	Blood examined immediately after death; did not show micrococci; the marrow of bone contained a moderate quantity of micrococci; distinct pseudo-membrane at seat of inoculation; micrococci in liver and kidneys.
286	June 11	Young albino rabbit.	Inoculated with matter from rabbit Exp. 239 in back.	Died May 17 ..	Micrococci in blood in cells and free; local lesion perfect; micrococci in kidneys.
287	June 11	Gray rabbit, moderate size.	Inoculated in thigh ...	Died May 16..	Same as last.
288	June 11	Small black rabbit.	Same as last	....do	Do.

These six experiments are abundantly sufficient to show that the micrococcus present in the pus of diffuse cellulitis does not lose its power by passing through the system of the rabbit, and that the membrane it invokes at the seat of inoculation is possessed of a deadly virulency.

The facts which have been established in the present research prove that the micrococcus of sloughing wounds, and of the so-called phlegmenous erysipelas, agrees in its physical, chemical, and pathological properties with that of diphtheria.

As we have submitted *Micrococcus septicus* and *M. diphtheriticus* to all known tests, optical, chemical, and vital, and found them always to respond alike, we conclude that the two organisms are specifically one, and that diphtheria is a septic sore throat, or is simply what it was called a century ago, *putrid sore throat, with or without a secondary constitutional septicæmia.*

The exceedingly pressing question whether the micrococci we have found in malignant measles, scarlatina, puerperal fever, &c., is the *M. septicus*, we are not prepared to answer positively. We have not practiced inoculation upon the lower animals, but the microscope and chemical reagents certainly indicate an identity. There is an explanation of the known facts which we offer with reserve as probable, though not positively proven.

In our examinations of the blood in the various diseases under discussion, we have never detected the micrococci before the second day of severe illness, and very rarely before the fourth day. We think that we have proven that the *M. septicus* is always about the human body in an inert state. It is very possible that in the conditions of vital depression and diseased secretions such as occur during low fevers, the organism finds the conditions for its development, and commencing to grow invades the system and produces a secondary blood-poisoning or septicæmia. This theory gains in plausibility when it is borne in mind that in scarlet fever, measles, and puerperal fever, the diseases which we studied, there are local foci of inflammation in which all the conditions are present for the urging of the micrococcus into growth and malignancy. There is no anatomical difference between a scarlatina sore throat and that of true diphtheria; the micrococcus is as abundant in the scarlatinal exudate as it is in the diphtheritic, and what we know of its life history shows that it must, under such circumstances, develop and invade the system. In malignant measles there are severe catarrhal inflammations; in erysipelatous cellulitis abundant local foci. It is therefore extremely probable, from the facts we have been able to establish, that secondary esoteric septicæmias are exceedingly important in the history of various diseases, and that often the patient dies, not directly from the original disorder, but from the secondary septicæmia it has brought about.