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STUDIES IN THE PATHOLOGY OF DIPHTHERIA.

BY J. H. WRIGHT, M. D.



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STUDIES IN THE PATHOLOGY OF DIPHThERIA.

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THE bacteriological studies of diphtheria and the Klebs-Löffler bacillus, the results of which are here reported, have been carried on under the direction of Professor Councilman in the Sears Pathological Laboratory of the Harvard Medical School and to some extent, also, in the Pathological Laboratory of the Boston City Hospital.

Practically all of the material for these studies has been derived from the contagious wards of the Boston City Hospital, for the most part during the terms of service of Dr. F. H. Williams and Dr. E. M. Buckingham, or in the period extending from October, 1893, to April, 1894. The work has comprised five main sections, as follows:

I. A study of the virulence of the bacillus diphtheriæ in infections of the pharynx and air-passages of varying degrees of severity and duration.

II. The results of autopsies on diphtheria cases.

III. The pathological anatomy and bacteriology of experimental diphtheria.

IV. Cases of infection of various ulcerated surfaces and other inflammatory processes with the Klebs-Löffler bacillus, as well as some other instances in which it has been isolated.

V. On the morphology and biology of the Klebs-Löffler bacillus.

I.

A study of the virulence of the bacillus diphtheriæ in infections of the pharynx and air-passages of varying degrees of severity and duration.

The question as to whether the bacilli obtained from mild cases of diphtheria are as virulent for guinea-pigs as those derived from severe cases has received special attention at the hands of but a few investigators. The most important work on this question is that of Roux and Yersin.¹ These investigators inoculated guinea-pigs with cultures obtained from 40 fatal and 39 non-fatal cases, and compared the results. They concluded that, in general, there is a striking difference to be observed as to virulence toward guinea-pigs, between the bacilli from cases ending in death and those terminating in recovery, although the latter often furnish organisms of full virulence.

Escherich² in his recent work confirmed these results, finding that in general mild cases yield weakly virulent cultures, but that the exceptions to this rule are numerous. In an earlier publication,³ however, he had stated that he had noted no difference in the virulence of the bacilli from cases of different degrees of severity. The writers just quoted seem to be the only ones who have studied this point especially. On the other hand, the work of other investigators (Löffler,⁴ v. Hofman,⁵ Zarniko,⁶ Beck,⁷ Spronk,⁸ Welch and Abbott,⁹ and others) who have inoculated guinea-pigs with the Klebs-Löffler bacillus from numbers of diphtheria cases, seems to point to no such relationship between the clinical character of the case and the effect of the bacillus derived from it upon the animal.

Another aspect of the question of the virulence of the bacillus diphtheriæ, which has received more atten-

tion, is as to whether the organism loses its virulence by long-continued residence in the air-passages of diphtheria patients.

It has been well shown by the work of Janson,¹⁰ Heubner,¹¹ Tobiesen¹² and Morse¹³ that the bacillus may persist in the throat long after convalescence has been established. The virulence of the organism in these cases of recovery has been found by Löffler,¹⁴ Heubner,¹¹ Tobiesen¹² and Escherich² to be undiminished, while, on the other hand, Roux and Yersin¹ have stated that a progressive diminution of virulence may be observed the longer the organism remains in the throat. The last named have recorded five cases in which virulent bacilli gradually gave place to non-virulent forms in the course of days and weeks.

A more extensive piece of work on this point is that of Tobiesen,¹² in which the bacilli from 19 convalescent diphtheria cases, after their discharge from the hospital, were inoculated into guinea-pigs. Of the 19 cases, 16 were found to still furnish bacilli which killed the guinea-pigs, with the characteristic lesions at the autopsy; while on the animals inoculated from the 3 remaining cases, 2 eventually died and 1 developed paralysis.

From all the foregoing statements it would appear that in general no constant relationship can be made out between the virulence of the bacillus towards animals, and the symptoms presented by the cases from which they were derived. Having at our disposal a large number of cases of diphtheria in the contagious wards of the Boston City Hospital, it was determined to again test this question of the virulence of the bacillus in the different types of cases above referred to. With this end in view two series of cases have been studied. The first and larger series comprises 59 cases of various degrees of severity from which the cultures have been taken at an early period of the disease when the organism could have been considered as not having been present a long time in the throat. These cultures have been inoculated into 114 guinea-pigs to determine their virulence. The second series comprises 23 cases in which the bacilli had been present in the throat or nose for a longer or shorter period of time when inoculated into guinea-pigs. In this series, 35 animals were used.

The methods used in the investigation have been as follows: The material for cultures has been obtained, in most of the cases, by touching the mucous membrane of the nose or throat with sterilized cotton "swabs." The "swab" consists of a piece of stiff wire, about five inches long, upon one end of which a small pledget of absorbent cotton has been wound. The swab thus made is placed in a test-tube, which is stoppered with cotton and the whole sterilized by heat before using. A large number of swabs prepared in this way are kept at hand in the hospital laboratory ready for use, and may be carried to and from the wards without danger of contamination.

The culture medium used for growing discrete colonies of the organism from which to prepare pure cultures has been coagulated beef-blood serum, containing one part in four of one-per-cent. sugar bouillon (Löffler's mixture). The mixture of blood serum and bouillon is coagulated in test-tubes by dry heat so as to form "slants," and then sterilized by steam in three successive days, as is done with ordinary media. On this culture medium the diphtheria bacilli grow vigorously and with characteristic morphology.



To obtain colonies of the bacillus from which to prepare pure cultures, some of the material secured on the swab in the manner above described, is applied over the surface of two or more tubes, and these are then placed in the incubator for development. After about eighteen to forty-eight hours, from the discrete colonies which have appeared, pure cultures may be made for inoculation. The cultures used and the methods employed in the inoculations have varied. With a few exceptions, which will be found duly noted in the detailed reports of the work, bouillon and agar cultures have been inoculated which have been about twenty-four hours in the incubator. In the majority of cases cultures in one-per-cent. grape-sugar bouillon of neutral to slightly alkaline reaction and colored with litmus, have been injected into the tissues of the abdominal wall in quantities less than one cubic centimetre by means of a hypodermatic syringe.

In the same manner other fluid cultures and suspensions in sterilized water of the twenty-four hours' growth of agar cultures have been injected. The method of direct inoculation from agar cultures by the platinum loop or wire through a wound on the abdominal wall has also been used in some instances. In order to avoid as much as possible any attenuation of the organism by cultivation, the cultures used for inoculation in the first mentioned series of 59 cases, have all, with few exceptions, been of the second generation from the patient.

The limitation of the cultures used in these cases for testing the virulence to those of the second generation, has greatly increased the necessary labor of the investigation, on account of the numerous failures to obtain pure cultures within so few generations. In the second series of 23 cases, however, the cultures used have not been so limited as to the number of generations. The usual number has been three; and, unless otherwise specified in the tables of results, the cultures used have been that number of generations from the patient. In both series of cases two animals, as a rule, have been inoculated from the same case, and in many cases with the same culture. Proceeding in the manner above described, the study of two series of cases before mentioned has been carried out.

In the subjoined tables are given some of the details and the results of the work. In them are to be found the name and the age of the patient, the size and weight of the animals when known, the manner of inoculation, its results, and any remarks thought necessary. In addition to these, in the table of the second series, comprising those cases in which the bacillus has been present for a longer or shorter time, the number of days which have passed since it was first demonstrated to be present, up to the date of the taking of the cultures for inoculation, is given for each case in a separate column.

The first series of 59 cases has been divided into four groups comprising the fatal, severe, medium severe, and mild cases. In comparing and discussing the results obtained from the study of both series of cases, we shall consider as indicating the virulence of the organism derived from the case, only the shortest time in which an animal died in those cases where more than one was inoculated. The sooner that death followed the inoculation, the more virulent will be considered the culture which was inoculated.

In general it may be said that a full virulent culture is one which causes the death of a guinea-pig within

three days or less, a culture of medium virulence one which causes the death of the animal in from three to five days. Cultures which only produce local necrosis and ulceration or death after a greater number of days may be considered as of slight virulence. The death of the animal within forty-eight hours or less after the inoculation, indicates a very high degree of virulence in the culture inoculated.

This is based upon the assumption which nearly all writers on experimental diphtheria seem to have adopted, namely, that the susceptibility of the guinea-pig to infection with the bacillus diphtheriæ is a nearly constant quantity. If this be true, and if the rapidity of the death of the animal be a measure of the degree of virulence of the organism, we should find that the two or more animals inoculated simultaneously in the abdominal wall with the bacilli from a given case would die very nearly at the same time. In many cases this has been observed, but a glance at the tabulated results will show in this and other instances considerable variation in the times of the survival of animals inoculated from the same case as well as from similar cases; so that we are inclined to doubt the entire correctness of the assumption of a constant susceptibility.

These variations are apparently more frequent in those cases in which different modes of inoculation have been used than in those in which both animals have been inoculated from the same culture and in the same manner, but this circumstance does not suffice to explain all the differences in results observed.

To account for these differences, it does not seem unreasonable to suppose that guinea-pigs vary in their degree of susceptibility to this infection, and this variation in susceptibility may be considered as playing a considerable part in the production of the irregularities in the effects of the inoculation.

It would appear, however, that in general, inoculation with bouillon cultures is more rapidly fatal than the inoculation of the growth from agar cultures. The observations on this point are not altogether satisfactory, for it is very probable that in some few cases confusion has arisen as to which animal was inoculated with the bouillon culture and which from the agar, but the evidence is on the whole sufficiently trustworthy to warrant the general statement. The difference between the forms of culture is especially apparent in those cases in which the animal, after recovery from an inoculation with an agar culture, has rapidly succumbed to a bouillon culture of the same origin as the first, notwithstanding the fact that the organism had been in cultivation for a considerable time and so subject to the chances of attenuation.

It is not probable that this greater virulence of the bouillon culture is due to an intoxication with the poisonous products of the bacillus preformed in the culture and in solution. That in cultures so young as those here employed and injected in the quantities noted, no effective amount of the poison of diphtheria exists is clearly shown by the results of those who have studied the toxalbumin of the bacillus diphtheriæ.

To determine for ourselves, however, whether the fluid of twenty-four-hour bouillon cultures contains any toxic substance, we have in several instances injected the sterile filtrates of such cultures in the same manner as for bouillon cultures and in greater quantities, but never with any noticeable effect on the animal. Two of these experiments were essentially as follows: Two-tenths of a cubic centimetre of a twenty-four-hour sugar

INOCULATIONS WITH THE BACILLUS DIPHTHERIÆ.

FATAL CASES.

Name.	Age.	Weight (grams) or size of guin. pigs.	Mode of inoculation.	Result of inoculation.	Remarks.
T. M.	6 years	285	.3 c. c. Sugar bouillon (litmus)	Death, 44 days	
C. C.	10 years	290	.2 c. c. Sugar bouillon (litmus)	Death, 36 hours	
J. M.	3 years	290	.2 c. c. Sugar bouillon (litmus)	Paralysis. Killed after 3 wks.	
H. W.	2 years	370	.2 c. c. Sugar bouillon (litmus)	Death, 34 days	
L. C.	13 years	290	.2 c. c. Sugar bouillon (litmus)	Death, 48 hours	
C. K.	11 years	290	.2 c. c. Sugar bouillon (litmus)	Death, 48 hours	
R. T.	2½ years	275	.2 c. c. Sugar bouillon (litmus)	Death, 36 hours	
E. K.	2½ years	340	.2 c. c. Sugar bouillon (litmus)	Death, 48 hours	
K. B.	3 years	270	.2 c. c. Sugar bouillon (litmus)	Death, 36 hours	
J. T.	8 years	240	.2 c. c. Sugar bouillon (litmus)	Recovered. Ulceration	
M. L.	4 years	240	.2 c. c. Sugar bouillon (litmus)	Death, 36 hours	
B. L.	3 yrs. 3 mos.	215	.2 c. c. Sugar bouillon (litmus)	Recovered	
R. R.	5 years	Small	.1 c. c. Sugar bouillon (litmus)	Death, 72 hours	Bacillus under cultivation more than 4 weeks when inoculated into second guinea-pig.
M. D.	2 years	Small	.1 c. c. Sugar bouillon (litmus)	Death, 4 days	Scarlet fever coincident.
P. D.	4 years	Small	.1 c. c. Sugar bouillon (litmus)	Death, 4 days	Scarlet fever coincident.
G. B.	9 years	Medium size	.1 c. c. Glycerine bouillon	Death, 36 hours	Scarlet fever coincident.
A. G.	2 years	Medium size	.1 c. c. Glycerine bouillon	Death, 48 hours	Scarlet fever coincident.
L. G.	7 years	280	.25 c. c. Sugar bouillon (litmus)	Death, 72 hours	Scarlet fever coincident.
M. C.	4 years	Well grown	.1 c. c. Glycerine bouillon	Death, 36 hours	Culture from membrane cast of trachea and bronchi.
B. H.	8½ years	Large	Agar culture loop	Death, 36 hours	Cultures from tracheal pseudo-membrane.
T. K.	4 years	Large	Agar culture loop	Death, 34 days	
E. E.	1 yr. 10 mos.	Small	.1 c. c. Sugar bouillon (litmus)	Death, 24 hours	
A. R.	2½ years	Medium size	.1 c. c. Sugar bouillon (litmus)	Death, 48 hours	Two agar cultures used.
E. H.	3 yrs. 5 mos.5 c. c. Water susp. agar cult.	Death, 36 hours	
E. F.	7 years5 c. c. Water susp. agar cult.	Death, 24 days	
J. R.	3 years5 c. c. Water susp. agar cult.	Death, 12-14 days	
E. R.	13 months5 c. c. Water susp. agar cult.	Recovered and reinoculated	The bacillus had been in cultures about 6 weeks when reinoculated.
B. M.	21 years	275	.2 c. c. Sugar bouillon (litmus)	Death, 48 hours	Pneumonia.

SEVERE CASES.

Name.	Age.	Weight (grams) or size of guin. pigs.	Mode of inoculation.	Result of inoculation.	Remarks.
M. M.	4 years	225	.2 c. c. Sugar bouillon (litmus)	Death, 36 hours	
Dr. T.	26 years	250	.2 c. c. Sugar bouillon (litmus)	Death, 37 days	No marked lesions at autopsy. Culture third gen.
Dr. B.	41 years	230	.3 c. c. Sugar bouillon (litmus)	Death, 36 hours	
G. M.	13 years	230	.2 c. c. Sugar bouillon (litmus)	Death, 34 days	
Dr. S.	28 years	200	.2 c. c. Sugar bouillon (litmus)	Death, 72 hours	
A. McD.	20 years	350	.5 c. c. Sugar bouillon (litmus)	Death, 36 hours	
L.	8 years	Well grown	.1 c. c. Glycerine bouillon	Death, 36 hours	Ulceration of throat. Reincoc. Survived reinoculation.
A. C.	9 years	Medium size	.1 c. c. Sugar bouillon (litmus)	Death, 36 hours	Ulceration of throat. Reincoc. Survived reinoculation.

CASES OF MEDIUM SEVERITY.

Name.	Age.	Weight (grams) or size of guin. pigs.	Mode of inoculation.	Result of inoculation.	Remarks.
F. G.	4 years	350	.3 c. c. Sugar bouillon (litmus)	Death, 3 days	Scarlet fever coincident.
G. G.	5 years	210	.2 c. c. Sugar bouillon (litmus)	Death, 72 hours	
F. B.	4 years	350	.5 c. c. Sugar bouillon (litmus)	Death, 48 hours	
W. McL.	6 years	435	.5 c. c. Sugar bouillon (litmus)	Death, 64 days	
P. W.	11 months	280	.2 c. c. Sugar bouillon (litmus)	Death, 3 days	Scarlet fever coincident. K.-L. bacilli in throat 10 days when this culture was taken.
E. M.	25 years	290	.5 c. c. Sugar bouillon (litmus)	Recovered	
E. G.	10 years	Medium size	.1 c. c. Water susp. agar cult.	Death, 8½ days	
D. G.	14 years	Medium size	.1 c. c. Glycerine bouillon	Death, 48 hours	Scarlet fever coincident. Gain-pig that recovered was reinoculated after about 5 wks.
M. K.	17 years	Medium size	.1 c. c. Sugar bouillon (litmus)	Recovered. Reinoculated	Both animals reinoculated with 72 hours glycerine bouillon culture after 2 weeks.
E. D.	33 years	Large	.1 c. c. Glycerine bouillon	Death, 72 hours	
F. M.	64 years	Small	Agar culture loop	Death, 36 hours	
M. S.	6 years	Large	.1 c. c. Bouillon	Death, 48 hours	Scarlet fever coincident.

MILD CASES.

Name.	Age.	Weight (grams) or size of guin. pigs.	Mode of inoculation.	Result of inoculation.	Remarks.
W. N.	32 years	360	.3 c. c. Sugar bouillon (litmus)	Death, 17 days	Culture in 3d gen.
B.	30 years	Large	Agar culture loop	Ulceration. Recovered	The animal which recovered was reinoculated after 2 weeks.
S. M.	50 years	Small	.1 c. c. Glycerine bouillon	Death, 72 hours	
S. R.	27 years5 c. c. Water susp. agar cult.	Death, 64 days	
V. P.	3 years5 c. c. Bouillon	Recovered. Reinoculated	Culture 72 hrs. old. Reinoculated about 6 weeks after first inoc.
J. C.	2 years5 c. c. Water susp. agar cult.	Death, 48 hours	
W. M.	2 years5 c. c. Water susp. agar cult.	Death, 48 hours	
N. D.	5 years5 c. c. Water susp. agar cult.	Death, 71 days	
W. P.	7 years5 c. c. Water susp. agar cult.	Death, 36 hours	
W. M.	24 years5 c. c. Water susp. agar cult.	Death, 72 hours	
J. L.	6 years5 c. c. Water susp. agar cult.	Death, 72 hours	

CASES IN WHICH THE BACILLUS HAD BEEN PRESENT FOR A LONGER OR SHORTER PERIOD OF TIME.

Name.	Age.	Days K.-L. pres.	Weight (grams) or size of guin. pig.	Mode of inoculation.	Result of inoculation.	Remarks.
I. T.	1 year	42	210	.2 c. c. Sugar bouillon (litmus)	Death, 72 hours	K.-L. bacilli in nose, never in throat. Cult. 2d gen. Medium severity, but eventually fatal.
F. H.	3 years	51	200	.2 c. c. Sugar bouillon (litmus)	Death, 48 hours	Main symptom coryza. Cult. 48 hours.
P. W.	13 mos.	49	200	.2 c. c. Sugar bouillon (litmus)	Death, 6 days	No lesions in animal at autopsy. Medium severity.
G. C.	6 years	30	Well grown	.1 c. c. Glycerine bouillon	Death, 36 hours	
F. B.	4 years	39	290	.3 c. c. Sugar bouillon (litmus)	Death, 36 hours	Culture 2d gen.
F. K.	5 years	29	330	.3 c. c. Sugar bouillon (litmus)	Ulceration, killed	Killed after 25 days. Medium severity.
M. C.	4 years	25	310	.3 c. c. Sugar bouillon (litmus)	Death, 38 days	No lesions at autopsy.
T. H.	25 years	18	225	.5 c. c. Sugar bouillon (litmus)	Death, 36 hours	
B. R.	27 years	27	265	.2 c. c. Sugar bouillon (litmus)	Recovered	
M. McD.	19 years	21	240	.3 c. c. Sugar bouillon (litmus)	Death, 4 days	Survived a second inoculation.
H. U.	5 years	26	210	.2 c. c. Sugar bouillon (litmus)	Death, 6 days	
Dr. P.	25 years	21	235	.25 c. c. Sugar bouillon (litmus)	Death, 36 hours	
Dr. T.	26 years	26	310	.25 c. c. Sugar bouillon (litmus)	Death, 33 days	Severe case.
W. T.	21 years	11	290	.3 c. c. Sugar bouillon (litmus)	Death, 6½ days	
W. M.	2½ years	14	180	.3 c. c. Sugar bouillon (litmus)	Death, 36½ days	No marked lesions at autopsy.
Dr. S.	26 years	19	280	.2 c. c. Sugar bouillon (litmus)	Death, 72 hours	Med. severity. 1st animal, 48 hrs., cult. 4th gen; 2d animal, 24 hrs., cult. 6th gen.
D. T.	5 years	16	250	.3 c. c. Sugar bouillon (litmus)	Death, 72 hours	
M. S.	7 years	14	240	.2 c. c. Sugar bouillon (litmus)	Recovered	Cult. of 2d gen. No lesions at autopsy. Culture of 3d gen.
G. P.	7 years	10	270	.2 c. c. Sugar bouillon (litmus)	Death, 29½ days	
H. C.	1½ years	8	280	.3 c. c. Sugar bouillon (litmus)	Death, 72 hours	No lesions at autopsy.
M. R.	6 years	9	210	.2 c. c. Sugar bouillon (litmus)	Death, 1½ days	
I. McL.	5 years	35	210	.2 c. c. Sugar bouillon (litmus)	Death, 8 weeks	Severe case.
J. S.	5 years	?	Large	.1 c. c. Water susp. agar cult.	Death, 95 hours	Med. severity. K.-L. bacilli present in nose when the culture was taken.

bouillon culture, two generations removed from the case of diphtheria, was injected in the usual manner into a guinea-pig to test the virulence. At the same time a portion of the culture was filtered through unglazed porcelain, and nine-tenths of a cubic centimetre of the sterile filtrate injected into another guinea-pig in the same way as the bouillon culture. Likewise the culture from another case of diphtheria was tested and the filtrate injected, but in this second case the quantity of filtrate injected into the abdominal wall was one cubic centimetre, and an additional cubic centimetre was thrown into the peritoneal cavity. Both of the animals inoculated with the bouillon cultures died within thirty-six hours, while those in which the filtrate had been injected in comparatively large quantities showed no ill effects.

It is clearly evident, therefore, that the toxic effects of the bouillon cultures here used have little to do with their apparently greater activity. Furthermore, as to whether a more rapid death follows the injection of a larger than a smaller quantity of the same culture, we do not care to express a positive opinion from the data at hand, for it is uncertain in some cases which animal received the larger quantity. It may be said, however, considering the results of inoculation of bouillon cultures as a whole, that two-tenths of a cubic centimetre seems to be quite as effective as one cubic centimetre.

The results of the study of the 59 cases of the first series may be briefly summarized as follows, if we apply the criteria of virulence above alluded to:

Of this number 28 were fatal and 31 ended in recovery.

Of the 28 fatal cases, cultures from 22, or 79 per cent., caused the death of at least one animal within three days, while of the 31 non-fatal cases, those of 23, or 74 per cent., had this effect. What may be considered as full virulent cultures were therefore only a little less frequently found among the non-fatal cases than among the fatal cases.

Of the 40 fatal cases of Roux and Yersin all, or 100 per cent., killed the animal within four days. Comparing our 28 fatal cases with these, we find that 25, or 89 per cent. of the cases gave cultures of this degree of virulence. The difference, therefore, between the fatal cases of Roux and Yersin and the similar cases of our series is represented by 100 per cent. in the former and 89 per cent. in the latter, of fairly virulent organisms. Of the 39 non-fatal cases of the above-named writers, 17, or 44 per cent., killed the animal in less than three days, while in our 31 cases, 23, or 74 per cent., had that result.

It cannot be said, therefore, that our results are completely in harmony with the classic studies of Roux and Yersin on diphtheria.

We may also combine the different groups of our first series in other ways, and institute comparisons. Comparing the fatal and severe cases together, on the one hand, with the mild and medium severe cases combined, on the other hand, we find that of the 36 fatal and severe cases, 28, or 78 per cent., killed a guinea-pig within three days, while of the 23 cases of mild and medium severity, 17, or 74 per cent., did so. The relation of the two combinations as to the frequency with which full virulent bacilli are found among them may therefore be expressed by the numbers 78 and 74, apparently indicating that full virulent cultures are not more frequently obtained from fatal and severe

cases than from the others. Again, considering the mild cases by themselves, it is found that 7 of the 11, or 64 per cent., as compared with 79 per cent. of the fatal cases, or 78 per cent. of the fatal and severe cases combined, gave cultures which caused the death of the animal within three days, and were therefore full virulent.

In making these comparisons between the different classes of cases, we have of course based them upon the percentage among them of what may be considered as quite virulent organisms and have not considered the cases which have yielded cultures of medium or slight virulence. It has been thought best to limit the comparisons to this point in order to avoid confusion.

The study of the second series of 23 cases, in which the bacilli have been present for from 8 to 51 days in the throat or nose, has been rendered possible by the rule of the Boston City Hospital, that no diphtheria case can be discharged until cultures from the throat and nose show the absence of Klebs-Löffler bacilli, although the case be otherwise fully recovered. By consulting the records of the routine bacteriological work on diphtheria carried on in the pathological laboratory in the same institution, we have been enabled to give in all of the cases, except one, the number of days which have passed from the date of the first demonstration of the organism by cultures in the throat or nose to the date of the taking of the culture for inoculation.

In the table of the results of our study of this series, the culture used is to be considered as of the third generation from the case, unless otherwise stated. These cases, moreover, unless otherwise specified, have been of mild character originally. The result of the work of this series may be briefly summarized as follows: Of the 23 cases, cultures from 11 have caused the death of the guinea-pig in less than three days, or 48 per cent. gave full virulent bacilli. It is interesting to note that in 7 of the 11 virulent cases the presence of the bacilli in the throat or nose had first been shown by cultures 26 to 51 days before the time that the culture inoculated was taken.

It will be seen, however, that this series, considered as a whole, shows fewer virulent cultures than the other series of 59 cases, the percentage in these persistent cases being 48 per cent. as compared with the 78 per cent. of the fatal and severe cases or the 74 per cent. of the mild and medium cases.

Whether these differences are due to any attenuation of the organism in this second series of cases, owing to the slightly greater number of generations through which it has been cultivated, we do not know. It seems, however, to be a generally accepted fact that the bacillus diphtheriæ is not easily attenuated by cultivation; and the general tendency of our own experience is in this direction, as will be seen from the results in some of the instances before alluded to, in which reinoculations of an animal have been made with a fresh culture of the same bacillus as the first inoculation. In some of these instances the organism has proven virulent although it had been under cultivation for some time. It may be mentioned that among the series of persistent cases are four cases, the bacilli from which had previously been inoculated and the results included in the first series. In two of these four cases the culture had been taken for inoculation respectively 33 and 39 days before the taking of the

culture of the present series. Both the earlier and later cultures in these two cases killed at least one animal in forty-eight hours, one of them with the later culture causing the death of the guinea-pig in thirty-six hours. Of the other two cases of the four above mentioned, one seemed to show some diminution in the virulence of the bacillus, while the fourth case on both occasions seemed to yield organisms of little virulence.

If the results of this study of the cases of the second series be compared with those of Roux and Yersin and of Tobiesen on the same class of cases, they will be found to be totally at variance with those of the former, but to agree fairly well with those of the last mentioned. Tobiesen¹² obtained from 16 out of 19 convalescent cases cultures which caused the death of guinea-pigs with characteristic lesions, while Roux and Yersin¹ found that in similar cases the occurrence of virulent bacilli was the exception, and believed that a progressive attenuation of virulence took place.

Considering now the whole number of 82 cases in both series, we find that but one case seemed to furnish organisms of complete innocuousness toward guinea-pigs. This case was a fatal one, and in neither of the two animals inoculated was any effect noted. There are, however, 12 cases which deserve special attention, as the bacilli from them showed little virulence. In 2 of these cases the animal developed a characteristic induration and ulceration at the seat of inoculation. One was purposely killed, the other survived. In 6 others of these cases the animals died from 4½ to about 54 days after inoculation, with very slight lesions (or none at all) observed at the autopsy. Cultures from the seat of inoculation were made at 5 of the autopsies, but in only one of them was the Klebs-Löffler bacillus recovered. This animal had been inoculated about 38 days before. In the remaining 4 cases the animals died in from 14 to 31½ days with lesions of experimental diphtheria, and the bacilli were removed from the seat of inoculation. Of one of these 4 cases it should be explained that lesions were present in one of the animals inoculated, but not in the other or only in slight degree, and that the bacilli were found at the seat of inoculation in the latter, but not in the former. The case has, therefore, been considered as one in which lesions were produced, and in which the bacilli were recovered from the seat of inoculation.

It may be seen from this description that of these 12 cases with bacilli of little virulence, 6 yielded bacilli which called forth more or less of a characteristic reaction in the animals, either locally or in the organs.

Finally, we may state that, with the exception of these 12 cases, from all of the remaining cases bacilli were obtained which killed at least one guinea-pig within ten days.

From our study of these 82 cases the following general statements may be made:

- (1) That there is practically no difference in virulence to be observed between the bacilli derived from severe and mild cases of diphtheria.
- (2) That cases in which the Klebs-Löffler bacilli have been present for a longer or shorter period of time, furnish fewer virulent cultures than cases of recent beginning.
- (3) That the Klebs-Löffler bacillus does not, as a rule, lose its virulence by long-continued residence in the pharynx and air-passages.
- (4) That the Klebs-Löffler bacillus exists with all

degrees of virulence down to innocuousness, as far as is shown by the results of its inoculation into guinea-pigs, and that the intensity of the reaction in the animal bears no constant relation to the symptoms presented by the case from which it was derived.

(5) That there is no relation to be observed between the age or sex of the patient and the virulence of the bacillus.

In conclusion, the writer desires to express his acknowledgments to Dr. Francis H. Williams for valuable clinical data on some of the cases. The thanks of the writer are also due to Dr. W. H. Prescott for kind assistance in this and other portions of the work.

II.

The results of fourteen autopsies on diphtheria cases.

The autopsies on cases of diphtheria which are here reported have been performed, unless otherwise specified, at the Boston City Hospital during the period extending from November, 1893, to May, 1894. The chief interest in this work attaches to the results of the bacteriological examination of the organs.

Up to the time of the well-known work of Frosch¹⁶ it was generally believed that the bacillus diphtheriæ never invaded the internal viscera, but was only to be found in the local inflammatory lesions. In 10 or 15 autopsies on this disease, this investigator, by using large amounts of material for each culture, could demonstrate the presence of the bacillus either in the blood or in some of the organs. One of the most frequent places in which it was found was in the pneumonic areas of the lungs. Previous to the work of Frosch but a very few instances are on record in which the Klebs-Löffler bacillus has been met with in these situations. One of these is its occurrence in the spleen in a case reported by Kolisko and Paltauf.¹⁶ In the cervical lymph-glands the bacillus has been observed by Schmorl¹⁷ in 7 out of 10 cases. Recently, Booker¹⁸ obtained it in cultures from the spleen, submaxillary gland, lung, and blood of heart in a case of diphtheria.

In the broncho-pneumonia of diphtheria it seems to have escaped observation entirely in spite of the great amount of work which has been done on the bacteriology of this frequent complication of the disease, until Johnston¹⁸ found in it a single case, and Strelitz¹⁹ in one case among eight autopsies on diphtheria. In addition to these cases and those reported by Frosch, it has also been observed by Flexner²⁰ in one of the two cases examined by him.

It is apparent, therefore, that the principal points to be considered in these autopsies are the occurrence of the organism in the viscera in general and in the broncho-pneumonias in particular.

METHODS.

The medium generally employed for cultures at the autopsy has been the coagulated blood serum described in the first section of this report. The cultures were, in general, made by spreading over the surface of this coagulated serum in test-tubes as large an amount of the tissue or fluid as would adhere to the end of a piece of coarse, flattened platinum wire, previously sterilized in the flame of a Bunsen burner. The Klebs-Löffler bacilli are usually present in very small numbers in the organs, and we are inclined to attribute the comparative frequency with which we have met them

both to the use of the culture medium above mentioned, upon which they grow much more vigorously than upon the ordinary glycerine agar or similar material, and to the large amount of the fluid or tissue spread over the culture media. In the detailed reports of the results of the study of these cases which will be found below, it has been thought sufficient to only indicate the lesions presented at each autopsy by giving the anatomical diagnosis and any necessary remarks. In the bacteriological diagnosis of the cases the presence of the four organisms, the Klebs-Löffler bacillus, the streptococcus pyogenes, the pneumococcus (diplococcus lanceolatus) and the staphylococcus pyogenes aureus are in general the only organisms which have been considered. Other bacteria, including the bacillus coli communis, have developed in the cultures more or less frequently; but we have devoted little attention to these.

AUTOPSY I. By Dr. Wright. Fred W., aged eight years. Clinical diagnosis, scarlet fever. No bacteriological examination of throat during life, as there was no evidence of diphtheria.

Anatomical diagnosis: Inflammation of pharynx, larynx and trachea with muco-purulent exudation; slight ulceration of epiglottis; broncho-pneumonia; congestion and edema of lungs; acute splenic tumor; acute parenchymatous degeneration of liver and kidneys; acute lymphadenitis.

Bacteriological diagnosis by cultures: Klebs-Löffler bacilli in exudation of pharynx and ulcer of epiglottis; a culture from one lung contained a few Klebs-Löffler bacilli, also streptococci; pneumococci (diplococcus lanceolatus), staphylococci (aureus), and other organisms; cultures from spleen, kidney, liver, mesenteric gland and blood of heart were negative.

Two guinea-pigs were inoculated with .2 c. c. each of a twenty-four-hour bouillon culture of the third generation of the Klebs-Löffler bacilli from the pharynx. Both died in thirty-six hours, with the characteristic lesions at the autopsy. The bacilli were recovered by culture from the seat of inoculation in each animal, but from none of the organs.

A culture of the Klebs-Löffler bacilli found in the lung (sixth generation) was similarly inoculated into another guinea-pig in the quantity of .2 c. c. Death in seventy-two hours, with the characteristic lesions. Klebs-Löffler bacilli recovered by culture from seat of inoculation. Organs sterile.

This case is of interest as affording an instance of a fatal diphtheria without the presence of pseudomembrane.

AUTOPSY II. By Dr. Councilman. Isabella N., aged nineteen. Clinical diagnosis, typhoid fever and diphtheria. This case has been more fully reported by Dr. Morse in the reports of the Boston City Hospital.

Anatomical diagnosis: Diphtheritic inflammation of pharynx; diphtheritic ulceration of tonsil and larynx; tracheitis; typhoid ulceration of ilium and colon; heart thrombus; renal infarction; broncho-pneumonia, congestion, edema and bronchitis of lung; acute splenic tumor; acute lymphadenitis.

Bacteriological diagnosis by cultures: Klebs-Löffler bacilli and streptococci in tonsil and trachea. Typhoid bacilli and streptococci in heart thrombus, kidney infarction, liver and kidney. Typhoid bacilli in spleen and in gall-bladder. Streptococci in pleura and lung. Bacillus coli communis in mesenteric gland. Cultures from blood of heart and tracheal lymph-gland negative.

The Klebs-Löffler bacilli from the trachea were inoculated into two guinea-pigs. One of the animals died in forty-eight hours, and exhibited the characteristic lesions of experimental diphtheria at the autopsy; .5 to .7 c. c. of a twenty-four-hour bouillon culture was used for the inoculation of this animal. The other animal recovered, having been inoculated from a culture on agar.

This case is an example of poly-infection with the Klebs-Löffler bacilli, the streptococcus pyogenes and the typhoid bacillus. The last-named organism was distinguished from the bacillus coli communis by not producing gas bubbles in solid media, by having no effect on litmus milk and by not growing visibly on potato; also by the fact that it was actively motile and grew characteristically on gelatine.

It should be stated, however, that in the case of the kidney infarction the typhoid bacilli did not satisfy the requirements as to the culture on litmus milk.

AUTOPSY III. By Dr. Stokes. Sylvester P., aged ten months.

Anatomical diagnosis: fibrinous exudation on epiglottis; acute laryngitis; broncho-pneumonia of both lungs; acute lymphadenitis of cervical, bronchial and mesenteric glands; acute parenchymatous degeneration of liver and kidneys; acute splenic tumor.

Bacteriological diagnosis: Klebs-Löffler bacilli and streptococci in larynx, cervical lymph-gland and lung. Streptococci in heart-blood, spleen and bronchial lymph-gland. Streptococci and bacillus coli communis in kidney. Cultures from the liver and mesenteric lymph-gland negative.

With an agar culture of the Klebs-Löffler bacilli from the cervical lymph-gland two guinea-pigs were inoculated. One of the animals died in forty-eight hours with the characteristic lesions. At the autopsy of the animal, cultures were made as usual from the seat of inoculation and the organs. In the culture from the kidney one colony of Klebs-Löffler bacilli developed, and in the culture from the seat of inoculation the colonies of the organisms were numerous.

This case is an example of the frequent association which has been observed of a streptococcus septicemia along with the infection with the Klebs-Löffler bacillus.

AUTOPSY IV. By Dr. Wright. Edith T., aged four years, three months.

Anatomical diagnosis: diphtheritic inflammation of pharynx and larynx, acute lymphadenitis, acute splenic tumor, ecchymoses in right lung and in mucous membrane of stomach.

Bacteriological diagnosis by cultures: Klebs-Löffler bacilli in pharynx, tonsil and epiglottis. Klebs-Löffler bacilli and streptococci in right lung and in mucous membrane of stomach. Kidney, spleen, liver, right ventricle of heart, tracheal and mesenteric lymph-glands all sterile.

A guinea-pig, weight 210 gms. received .2 c. c. subcutaneously of a twenty-four-hour bouillon culture of the Klebs-Löffler bacilli from the lung. Death in forty-eight hours with the characteristic lesions, and the bacillus was recovered from the seat of inoculation, but found nowhere else.

The finding of Klebs-Löffler bacilli in the stomach has been reported by Löffler²¹ in a single case. In his case a diphtheritic inflammation of the stomach was observed, which was not apparent in the one here reported.

AUTOPSY V. By Dr. Councilman. Fred B., aged four years.

Anatomical diagnosis: Diphtheria and septicemia; erythema of skin; diphtheritic inflammation of pendulous palate and tonsils; acute lymphadenitis, with necrotic foci in cervical lymph-glands; edema of posterior tracheal tissues; acute nephritis.

Bacteriological diagnosis by cultures: Klebs-Löffler bacilli and streptococci in spleen, liver and cervical lymph-glands. Streptococci in kidney and blood of heart.

A guinea-pig weighing 210 gms. received subcutaneously .2 c. c. of a twenty-four-hour bouillon culture of the Klebs-Löffler bacillus found in the lung. Death in thirty-six hours with the usual lesions. In addition to being recovered from the seat of inoculation, the bacilli were also found in the cultures made from the blood, liver and spleen of the animal. The kidney was negative.

AUTOPSY VI. By Dr. Wright. Beatrice B., aged nine years.

Anatomical diagnosis: Diphtheritic inflammation of pharynx, tonsils, epiglottis and larynx; tracheitis; broncho-pneumonia of both lungs; acute lymphadenitis; acute splenic tumor; arterio-sclerosis of beginning of ascending aorta.

Bacteriological diagnosis: Klebs-Löffler bacilli in larynx, tonsil and lung; spleen, liver, kidney, heart-blood and mesenteric lymph-gland negative for either Klebs-Löffler bacilli or streptococci.

A twenty-four-hour bouillon culture of the bacillus from the tonsil and also from the lung killed two guinea-pigs in thirty-six hours, with the usual lesions. The quantity injected in each case was .2 c. c.

AUTOPSY VII. By Dr. Councilman. Charles Van G., aged three years.

Anatomical diagnosis: Diphtheritic inflammation of mucous membrane of nose and pharynx; slight broncho-pneumonia in both lungs; excoriations on face and nose.

Bacteriological diagnosis by cultures: Klebs-Löffler bacilli and streptococci in pharynx, nasal cavity and lung. Streptococci in blood of heart, in liver and in spleen. Kidney and cervical lymph-gland sterile. In mesenteric gland Klebs-Löffler bacilli. The culture from the excoriation on face showed the presence of Klebs-Löffler bacilli, and that from the nose Klebs-Löffler bacilli and streptococci.

AUTOPSY VIII. By Dr. Councilman. Child. Female.

Anatomical diagnosis: Operation wound of hip; abscess about hip-joint, involving ilium and retroperitoneal tissues; metastatic abscesses in kidney, lungs and left shoulder; diphtheritic inflammation of larynx, with necrosis and erosion of epiglottis; broncho-pneumonia and bronchitis.

Bacteriological diagnosis by cultures: Staphylococcus aureus in primary abscess, kidney, spleen and liver. Klebs-Löffler bacilli and staphylococcus pyogenes aureus in cultures from tonsil, epiglottis and lung (areas of broncho-pneumonia).

AUTOPSY IX. By Dr. Wright. Irene T., colored, aged one year. In contagious wards more than two months. Klebs-Löffler bacilli in cultures made from nose during seven or eight weeks. Never found in throat during life. *Vide* first case in the second series of preceding section.

Anatomical diagnosis: Extensive diphtheritic ulcer-

ation of epiglottis and larynx; diphtheritic inflammation of inferior turbinate bones and nasal cavities with fibrinous exudation; infarction of lung; acute degeneration of liver and kidneys; acute lymphadenitis; acute splenic tumor; slight pericardial effusion; otitis media and abscess of both mastoid bones.

Bacteriological diagnosis by cultures: Klebs-Löffler bacilli in ulcer of larynx and epiglottis and in bronchial lymph-gland. In lung infarction fairly numerous Klebs-Löffler bacilli and other bacteria. Streptococci in liver and spleen. Pneumococci in kidney (diplococcus lanceolatus). In blood of heart a few bacilli (coli communis?). Klebs-Löffler bacilli and streptococci in pus of mastoid abscess.

This case will again be referred to in a following section.

AUTOPSY X. By Dr. Councilman. Charles R., aged four years.

Anatomical diagnosis: Diphtheritic ulceration of pharynx and larynx; edema of pharynx; tracheitis; bronchitis; extensive broncho-pneumonia of right lung; erythema of skin; purulent conjunctivitis and keratitis.

Bacteriological diagnosis by cultures: Epiglottis, Klebs-Löffler bacilli and streptococci. Conjunctival pus, Klebs-Löffler bacilli and other bacteria. Lung, Klebs-Löffler bacilli, streptococci, pneumococci and other organisms. Edematous tissue with necrotic foci, behind left tonsil, streptococci. Kidney, streptococci, one colony; two to three colonies of staphylococcus aureus and other organisms. Spleen, very numerous colonies of various organisms, among them pneumococci.

AUTOPSY XI. By Dr. Councilman. Thomas M., aged six years. *Vide* list of fatal cases, Section 1. The bacilli taken from the throat during life were found virulent. This case will be referred to in another section.

Anatomical diagnosis: Diphtheritic inflammation of tonsils; diphtheritic inflammation and ulceration of trachea; broncho-pneumonia and edema of both lungs; acute parenchymatous degeneration; acute lymphadenitis; paronychia of toe.

Bacteriological diagnosis by cultures: In tonsil, trachea and bronchial lymph-gland, Klebs-Löffler bacilli, streptococci and staphylococcus pyogenes aureus. In lungs, Klebs-Löffler bacilli, streptococci, pneumococci, and in one lung also staphylococci pyogenes aureus.

In liver, Klebs-Löffler bacilli and streptococci. Streptococci in blood of heart and liver. Klebs-Löffler bacilli and staphylococcus pyogenes aureus in pus from paronychia. Right kidney, spleen and tracheal lymph-gland sterile; left kidney a very few colonies of the bacillus coli communis.

A guinea-pig weighing 200 gms. was inoculated with .2 c. c. of a twenty-four-hour bouillon culture of the Klebs-Löffler bacilli from the bronchial lymph-gland. No reaction was noted in the animal.

This case is noteworthy as being a fatal diphtheria in which a non-virulent organism was found at the autopsy while the Klebs-Löffler bacilli found during life were virulent.

AUTOPSY XII. By Dr. Wright. Annie O'T., aged one year.

Anatomical diagnosis: Diphtheritic inflammation of pharynx, larynx and trachea; broncho-pneumonia; ulceration of base of tongue; necrosis and ulceration of epiglottis; acute lymphadenitis; acute splenic tumor.

Bacteriological diagnosis by cultures: Klebs-Löffler bacilli in larynx, pharynx, bronchi of both lungs and in

small superficial ulcer on left hand. Streptococci and Klebs-Löffler bacilli in cervical lymph-gland and in one lung. Streptococci in the other lung. Kidney, spleen, liver and blood of heart, sterile.

A guinea-pig weighing 280 gms. was inoculated with .3 c. c. of a twenty-four-hour bouillon culture of the bacilli from a bronchus. Death after six days. The organism was found by culture in the blood and spleen of the animal, in addition to being found at the seat of inoculation.

Another guinea-pig weighing 245 gms. received .2 c. c. of a twenty-four-hour bouillon culture of the bacilli found in the lung. The animal survived for thirty-six days, with an ulceration at the seat of inoculation. The bacilli were recovered by cultures from the ulcerated tissue, but from none of the organs.

AUTOPSY XIII. By Dr. Councilman. Edward B. Clinical diagnosis, scarlet fever. The case had been recently operated upon for congenital hernia before entry into hospital.

Anatomical diagnosis: Erythema of skin; operation wound in abdominal wall with abscess formation; diphtheritic ulceration of pharynx and larynx; bronchitis; broncho-pneumonia of both lungs; acute degeneration of parenchymatous organs.

Bacteriological diagnosis by cultures: Larynx and pharynx, Klebs-Löffler bacilli. In one of these cultures also streptococci. Kidney, numerous streptococci. Spleen, numerous Klebs-Löffler bacilli and a few streptococci. Other organisms present. Liver, a few streptococci.

AUTOPSY XIV. By Dr. Wright. Sarah F., aged two and a half years.

Anatomical diagnosis: Diphtheritic inflammation of epiglottis, trachea and bronchi; pseudomembrane present in trachea about bifurcation and extending into bronchi; bronchitis; congestion of lungs; ecchymoses in mucous membrane of stomach; acute lymphadenitis; acute splenic tumor; acute parenchymatous degeneration; excoriations on leg and about anus and vulva.

Bacteriological diagnosis by cultures: Klebs-Löffler bacilli and staphylococcus pyogenes aureus in trachea, lung, liver, kidney, heart-blood, and in excoriations on leg. In addition to these the lung culture contained a few streptococci and pneumococci (?); that of the liver, streptococci; and the kidney culture, pneumococci. The spleen was sterile. In culture from tracheal lymph-gland a few streptococci and one colony of Klebs-Löffler bacilli. In the culture from mesenteric lymph-gland a very few Klebs-Löffler bacilli and other organisms.

The important points in the foregoing may be briefly summed up as follows:

Of the 14 autopsies the Klebs-Löffler bacillus has been found in the lung in 13 cases, in the liver in 3, in the spleen 2, in the cervical or bronchial lymph-glands in 5, in the kidney in 1, in the blood of heart in 1, and in the mesenteric lymph-glands in 2. It has been cultivated from the mucous membrane of the stomach in two instances and once from the edematous tissue behind the esophagus. In 7 of the 12 cases, or nearly 60 per cent., there has been a streptococcus septicemia in addition to the diphtheritic infection.

This seems to be another confirmation of the view of Barbier³³ and others that there are two distinct kinds of diphtheria: one, the toxic form or a monoinfection with the Klebs-Löffler bacillus; the other a polyinfection with that organism and the streptococcus.

In two other cases there has also been a similar polyinfection in which the staphylococcus pyogenes aureus has taken the place of the streptococcus in the septicemia. One of these septicemias, however, might be considered as having an infection-atrium in an abscess about the hip-joint, and one of the streptococcus septicemias an infection-atrium in an operation wound for congenital hernia.

In addition to these there is one other case of polyinfection (Autopsy X), in which there seems to have been a general infection with several organisms; but, owing to the lack of cultures from the liver and heart blood, nothing very definite can be said about it.

Broncho-pneumonia was present in 10 of the 14 autopsies. The occurrence of the Klebs-Löffler bacillus in the lung seems to be independent of the coincidence of this lesion, for it has been absent in one broncho-pneumonia and yet present where no pneumonic condition was made out.

In fact, we are inclined to explain the frequent occurrence of the bacillus in the cultures from the lung in the cases to its being present in the smaller bronchi—an explanation which Strelitz has made use of in the case reported by him. The organism most frequently found associated with the Klebs-Löffler bacillus in the lungs has been the streptococcus. These two have been present together in 9 cases, in 7 of which there was a pneumonic condition. The streptococcus has also been noted in pure culture in one pneumonia.

The pneumococcus (*diplococcus lanceolatus*) has been found much less frequently, appearing in but 3 or probably 4 of the 10 cases of pneumonia, and then always along with the streptococcus and Klebs-Löffler bacillus.

The staphylococcus pyogenes aureus has been observed in four of the pneumonias, once with the Klebs-Löffler bacillus alone, and three times in company with all three of the above mentioned organisms.

III.

The pathological anatomy and bacteriology of experimental diphtheria.

In the course of all of this work autopsies on guinea-pigs which have died after inoculation with the Klebs-Löffler bacillus have been made in 160 instances. This number includes two animals inoculated from an autopsy not included in our list. In the majority of cases notes have been taken with reference to the condition of the tissues at the seat of inoculation, the presence or absence of the characteristic subcutaneous edema, the presence of the acute lymphadenitis, of fluid in the thoracic cavity, of edema of the lungs, of necrotic foci in the liver and the condition of the suprarenal capsules.

These points embrace nearly all of the gross lesions which are observed in experimental diphtheria, and it has been considered worth while to give here in a statistical manner the results of our observations.

Local reaction of varying intensity has been observed in 145 of the 160 guinea-pigs. This has consisted in general of injection, edema, hemorrhage, fibrinous exudation and necrosis of the tissues of the abdominal wall. Great variation is observed in the character of the lesions at this point. In some cases nothing more than slight injection and a few slight hemorrhages are seen, while in others all of the conditions mentioned

may be present. In animals which have survived for some days the tissues at this point may be sometimes observed to be infiltrated with a dense, tough, brawny material extending over a considerable area and greatly increasing the thickness of the belly wall. Over this area the skin is tightly adherent. In 13 animals, including 2 which did not succumb to the effects of the inoculation, ulceration has been observed. In some of these the eschar has been several centimetres in diameter. The sloughing process seems to be preceded by an induration of the character above described in the tissues beneath.

The occurrence of the typical gelatinous edema of the subcutaneous tissues of the abdomen and thorax has been observed in a certain number of cases.

Local edema, more or less extensive, may be said to have been present in about 100 of the 160 animals.

Enlargement and usually more or less congestion of some or all of the lymph-glands of the inguinal and axillary regions has been noted in 142 of the autopsies. In some cases the glands have presented a hemorrhagic condition.

The lymph-glands at the brim of the pelvis in the retroperitoneal region and those of the mesentery seem to be usually affected along with the superficial ones. Congestion of the mesenteric glands is much less frequent, however, than in the case of the retroperitoneal glands.

Effusion of fluid into the thoracic cavity has been noted in 68 of the 160 autopsies. The fluid was almost invariably colorless and varied greatly in amount.

In some instances the thorax has apparently been completely filled. Edema of the lungs is recorded in 82 cases.

A characteristic lesion of experimental diphtheria is the presence of necrotic areas, visible to the naked eye in the liver. These lesions are not common, as will be apparent from the fact that they have occurred in about 49 instances, or 31 per cent., and their occurrence seems to bear no relation to the duration of the infection in the animal.

A more striking phenomenon, however, than any of the preceding, is the congestion or hemorrhagic condition of the suprarenal capsules. These have been found more or less red in color, instead of the normal bright yellow, in 130 of the 160 cases. This condition is almost invariably present in animals which have succumbed within a few days after inoculation; and in general it may be said that the combination of all the lesions enumerated above is to be more frequently observed in acute cases.

But one of the animals inoculated in the course of the work developed paralysis. This is in accord with the experience of Löffler, who seems to have observed it very rarely in the guinea-pigs inoculated by him.

In our study of the bacteriology of experimental diphtheria the main purpose has been to determine the frequency with which the Klebs-Löffler bacillus invaded the blood and internal organs of the inoculated animal. In this disease in the guinea-pig as well as in diphtheria in man, the bacillus has been considered as remaining at the point of invasion. The only observations of its occurrence in the organs of an inoculated guinea-pig which we have found in the literature is that of Zarniko,⁶ who mentions having met with it in a necrotic focus in the liver, and that of Abbott,²² who has reported the presence of the bacillus in certain lymphatic structures in the mesentery.

At the autopsies of the animals, as a matter of routine, cultures have been made from the seat of inoculation, blood of heart, liver, spleen and kidney. The coagulated blood serum previously described has been the medium employed for the purpose, with a few exceptions. As in the autopsies on man, rather more material than is usual has been commonly taken for each culture, by means of the coarse flattened platinum wire. In a few cases cultures have either not been made at all or only from some of the above-named situations. The numbers given below include cultures from three animals which have not died spontaneously, but have been purposely killed. The results may be briefly summarized as follows: among 155 livers from which cultures were made, 19 yielded Klebs-Löffler bacilli. Of 152 cultures from as many spleens, 15 contained the same organism. The bacillus also grew in 4 of 151 cultures from the kidney, and in 7 of 153 cultures from the blood of the heart.

The organism has therefore occurred most frequently in the liver and spleen, being found in the former in 12 per cent. of the autopsies. The colonies which develop in these cultures are, as a rule, few in number, rarely exceeding a half-dozen in a tube. The presence of the Klebs-Löffler bacilli in the viscera does not seem to bear any relation to the length of time the animal survived nor to the clinical character of the case from which the culture used for inoculation was derived, nor is its presence in the liver definitely associated with the occurrence of the foci of necrosis alluded to above. Moreover, we have no reason to believe that its presence in any internal organ has any effect *per se* upon the tissue of that organ.

The cultures from the seat of inoculation in 161 animals have contained the bacillus inoculated in all but 20. It may also be mentioned that from three guinea-pigs the organism has been recovered by culture from the seat of inoculation about thirty-one to thirty-eight days after the date of inoculation. The persistence of the bacillus in the tissues for this length of time is interesting.

IV.

Cases of infection of various ulcerated surfaces and inflammatory processes with the Klebs-Löffler bacillus, as well as some other instances in which it has been observed.

The occurrence of the Klebs-Löffler bacillus in other pathogenic processes than diphtheria has been rarely noted in the literature. The only other condition in which its presence has been repeatedly demonstrated is rhinitis fibrinosa. That this disease, however, is really to be regarded as a form of diphtheria of the nasal mucous membrane, has been shown by the work of Concetti,²³ Stamm,²⁴ Baginsky²⁵ and Abbott.²⁶

In the edematous tissue about tracheotomy wounds in diphtheria, Spronk²⁷ has found the Klebs-Löffler bacillus in three cases. It has also been observed by Neisser²⁸ in a superficial ulceration about the anus of a diphtheria case, and was proven virulent. Brunner²⁹ isolated it from three cases of inflammation of the fingers and from a case of phlegmon of the scrotum. The bacilli from only two of these were found to be virulent, the others yielding organisms of a mild degree of virulence. In all four cases the bacillus was accompanied by pus organisms.

In diphtheritic conjunctivitis its presence has been reported by Escherich,² by Babes,³⁰ by Elschieg³¹ and

by Kolisko and Paltauf¹⁶ in single cases. In the case of the first mentioned the organism was non-virulent. Three cases of the occurrence of the Klebs-Löffler bacillus in the pus of otitis media have been reported by Councilman,²² and one case of its occurrence in diphtheritis of the stomach by Löffler.²¹ Finally, Howard²³ has found it in pure cultures and in large number in the valvular vegetations, spleen and kidney of a case of ulcerative endocarditis. The organism here was found to be non-virulent. The writers named seem to comprise all those who have observed this organism in inflammatory processes other than diphtheria.

It will be noted that in most of the few cases in which inoculations have been made, the bacillus from these sources has exhibited little or no virulence. This lack of virulence, or rather the manifestation of only slight virulence, towards guinea-pigs has also been observed in some of our cases, as will be seen below.

In the study of cases of the occurrence of the Klebs-Löffler bacillus which are reported in this section, the same methods as those described in the preceding section have been employed.

When convenient the sterilized cotton "swabs" have been used for obtaining the material for culture. The bouillon cultures used for inoculation have been made in the one-per-cent. sugar bouillon colored with litmus, which was mentioned in the first section of this work. Cultures, have, as in the other cases, been made from the organs and the seat of inoculation of the animals which succumbed.

Some of the cases have been observed at the autopsies reported in the second section, but it has been thought better to reserve the special descriptions for this section.

CASE I. Fistula in ano. Wm. B., aged eighteen years. Admitted to contagious wards of the Boston City Hospital with diphtheria. Klebs-Löffler bacilli were found in throat by culture. The patient also had a fistula in ano. Some two weeks after admission active inflammation developed in this. The ulcerated surface exposed between the nates was about one inch square. The tissue about it was inflamed for two or three inches. The ulcer was covered with a greenish, slimy exudation, and eventually a false membrane was formed. This acute condition subsided after a time, and the patient also recovered from the diphtheria.

A culture from the ulcer showed the presence of Klebs-Löffler bacilli. These were found very virulent, the two animals dying in thirty-six and seventy-two hours respectively after inoculation with .2 c. c. and .4 c. c. of a twenty-four-hour bouillon culture. They exhibited the characteristic lesions, and the organism was recovered by culture from the seat of inoculation in both.

CASE II. Ulcer on arm. Winifred C., aged ten months. *Vide Case X.* Admitted to contagious wards with diphtheria. Klebs-Löffler bacilli demonstrated by culture. Some time after admission, from a vaccine pustule on left arm a deep gangrenous ulcer formed. A culture from this showed the presence of Klebs-Löffler bacilli.

Two guinea-pigs, weighing 210 and 260 gms. received respectively .2 and 1 c. c. of a twenty-four-hour bouillon culture of the third generation. Both survived from seven to eight days. At their autopsy, exudation and infiltration was found at the seat of inoculation. The only other marked lesion was edema of the lungs in each, as well as some glandular enlargement.

The Klebs-Löffler bacilli were recovered by cultures from the seat of inoculation in both, and from the liver, spleen and kidney of one. The cultures from the other animal showed the presence of the bacillus in the spleen alone.

CASE III. Excoriations on face. Charles Van G., aged three years. *Vide Autopsy VII.*

At the autopsy of this case, excoriations on face and nose were noted and cultures made from them. In the culture from the excoriation on the nose Klebs-Löffler bacilli and streptococci developed and in that of the excoriations on the face, Klebs-Löffler bacilli. No animal inoculations were made.

CASE IV. Excoriation on lip. Fred B., aged four years. *Vide Autopsy V.*

At the autopsy of this case a number of small excoriations were observed on the lip below the nasal opening. Klebs-Löffler bacilli developed in the culture made from these.

A guinea-pig weighing 425 gms. received .3 c. c. of a twenty-four-hour bouillon culture (third generation) of this bacillus subcutaneously. The animal died in forty-eight hours.

The usual appearances of acute experimental diphtheria were noted at the autopsy. The bacillus was recovered by culture from the seat of inoculation, but from nowhere else.

CASE V. Excoriation on leg. Sarah F., aged two and a half years. *Vide Autopsy XIV.*

At the autopsy of this case a slight excoriation over anterior aspect of the left tibia was observed and a culture was made from it. Klebs-Löffler bacilli and staphylococcus pyogenes aureus developed in this. No animal inoculation.

CASE VI. Ulcer on leg. Idella McL., aged five years. *Vide Section I, Series 2.* Admitted to the contagious wards with diphtheria. Klebs-Löffler bacilli present by culture. Some time after admission a deep undermining ulcer with brawny edges and grayish base formed on right leg. It was supposed to have originated from a puncture with a hypodermic syringe.

A culture on two occasions, several days apart, showed the presence of Klebs-Löffler bacilli. The bacilli obtained from the first culture did not cause the death of the animal until about twenty-nine days after inoculation. The guinea-pig weighed 190 gms. No definite lesions were found at the autopsy, and the bacillus was not recovered from the seat of inoculation. The second culture, however, furnished a virulent organism, killing a guinea-pig (weight 210 gms.) in thirty-six hours. The quantity inoculated in each case was .2 c. c. and a bouillon culture was used. The first animal was inoculated with a culture of the fifth generation and the second animal with one of the third generation. It may be possible that the discrepancy in the results of the inoculation may have been due to some attenuation of the organism which was obtained in the first instance. It may be added that the second animal showed characteristic lesions and the bacilli were recovered from the seat of inoculation but from none of the organs.

It is worthy of note that the bacilli from the nose of this case showed little or no virulence.

CASE VII. Ulcer on hand. Annie O'T., aged one year. *Vide Autopsy XII.*

At the autopsy of this case a small superficial ulcer less than one centimetre in diameter was observed on

the left hand. A culture made from this yielded Klebs-Löffler bacilli as well as other organisms.

A guinea-pig weighing 220 gms. was inoculated subcutaneously with .3 to .5 c. c. of a twenty-four-hour bouillon culture of the third generation. The animal survived four and a half days. Characteristic lesions were found at the autopsy. The blood of the heart as well as the seat of inoculation was found to contain Klebs-Löffler bacilli. In the culture from the former but one colony developed. The other organs were negative. It may be mentioned that the bacilli from the bronchus and from the lung of this case were also found to be of medium or slight virulence toward the guinea-pig, as may be seen by Autopsy XII.

CASE VIII. Otitis media. Pus from ear. Irene T., aged one year. *Vide* Autopsy IX and Case IX.

The otitis media with perforation of the tympanum occurred in the course of diphtheria. Klebs-Löffler bacilli had been present, by culture, in the nose for some time (*vide* Section I, Series 2). A moderate number of Klebs-Löffler bacilli and numerous streptococci were found to be present in cultures made during life from the thin but abundant purulent discharge from the ear.

A guinea-pig weighing 190 gms. was inoculated subcutaneously with .2 c. c. of a twenty-four-hour bouillon culture (third generation) of the Klebs-Löffler bacilli thus obtained. The animal died in thirty-six hours with characteristic lesions. The bacillus was recovered only from the seat of inoculation.

CASE IX. Mastoid abscess. Irene T. *Vide* Autopsy IX, *et supra*.

At the section the calvarium and brain were removed and the mastoid cells on both sides broken open. Both were found to contain creamy pus. A culture was made from the pus of the mastoid bone of the side opposite to that of the ear from which the culture mentioned above was taken (*intra vitam*). On this there developed Klebs-Löffler bacilli and streptococci. No animal inoculated.

CASE X. Otitis media (?). Pus from ear. Winifred C., aged ten months. *Vide* Case II.

Admitted to contagious wards with diphtheria. The presence of Klebs-Löffler bacilli was demonstrated by cultures. In the course of her stay in the ward a discharge from the ear was observed. This was found to contain Klebs-Löffler bacilli along with other organisms.

A guinea-pig of 340 gms. weight, which was inoculated with .3 c. c. of a twenty-four-hour bouillon culture, fifth generation, survived eleven and one-half days. Some of the lesions of experimental diphtheria were found at the autopsy, principally shown by a red color of the suprarenals. The bacillus was recovered from the seat of inoculation. As in the case of the bacillus from the ulceration on the arm of this same patient, this bacillus from the ear showed only a slight degree of virulence. Some attenuation by cultivation may have taken place, however, as it was inoculated in the fifth generation.

CASE XI. Purulent conjunctivitis with keratitis. Charles R., aged four years. *Vide* Autopsy X.

At the autopsy the right eye was observed to contain a considerable amount of pus beneath the lids, and the cornea was cloudy. A culture from the pus showed the presence of Klebs-Löffler bacilli in addition to pus organisms. No inoculation of an animal was made.

CASE XII. Thomas M., aged six years. *Vide* Autopsy XI.

At the autopsy a culture was taken from the paronychia of the toe. In this there developed Klebs-Löffler bacilli and numerous colonies of staphylococcus pyogenes aureus.

A guinea-pig weighing 200 gms. was inoculated with .2 c. c. of a twenty-four-hour bouillon culture, third generation, of the Klebs-Löffler bacilli thus obtained. The animal survived, as did also the guinea-pig inoculated with the Klebs-Löffler bacillus found in the bronchial gland. As has been stated elsewhere, virulent bacilli were found in the throat of this case during life. The organism inoculated was studied in cultures on blood serum, agar and litmus bouillon, and was found both in cultures and in morphology to agree with the typical Klebs-Löffler bacillus. The litmus bouillon was rapidly colored red, which, according to Escherich,² is only seen in cultures of the virulent bacillus.

CASE XIII. Acute ulcerative endocarditis. Autopsy by Dr. Councilman. Body, that of a young man.

Anatomical diagnosis. Acute ulcerative endocarditis, acute intracapillary glomerulo-nephritis.

Bacteriological diagnosis by cultures: Spleen, liver, both lungs, wall of left ventricle of heart, all negative. Aortic valve (vegetation), Klebs-Löffler bacilli (a half-dozen colonies), pneumococci, staphylococci, bacillus coli communis. Tricuspid valve, Klebs-Löffler bacilli (a half-dozen colonies), pneumococci and other organisms. Kidney, two typical colonies of typical Klebs-Löffler bacilli developed in the culture.

The Klebs-Löffler bacilli from the valves of each side of the heart were inoculated in bouillon cultures into four guinea-pigs. The quantities used varied from .3 or .4 c. c. to 1 c. c. All of the animals survived, the inoculation having little or no effect upon them even locally. The bacilli from each valve were also carefully studied in their growth in bouillon, agar, blood serum, potato and gelatine as well as in their morphology. In all these respects they were found to be identical with the Klebs-Löffler bacillus.

It may be mentioned also that they produced a rapid reddening of the litmus-sugar bouillon, which Escherich states is only done by virulent Klebs-Löffler bacilli. This red color was rapidly produced and persisted indefinitely. The agar cultures were never observed to show the brown color which the same writer regards as characteristic of the so-called pseudo-diphtheritic bacillus. The bacillus found in the kidney also changed the bouillon to a red color.

This case resembles very much the case of ulcerative endocarditis reported by Howard, which we have before alluded to. In that case a non-virulent Klebs-Löffler bacillus was found in the valvular vegetations and in the organs. Its identity with the true Klebs-Löffler bacillus in all respects except virulence was fully demonstrated.

CASE XIV. From a tuberculous lung. John S., aged twenty-six years. Autopsy by Dr. Councilman.

The case was essentially one of disseminated tuberculosis. The lungs were most affected. At the autopsy cultures were made as usual from all the organs. There was a tuberculous ulcer of the trachea from which no cultures were made. In the culture from the lung numerous colonies of Klebs-Löffler bacilli and streptococci developed, in addition to a few colonies of

staphylococcus aureus and other organisms. Streptococci and other organisms were present in the culture made from the spleen, while the kidney and liver were sterile.

The Klebs-Löffler bacillus from the culture from the lung was inoculated into a guinea-pig weighing 200 gms.; .5 c. c. of a twenty-four-hour bouillon culture of the second generation was used. Death in thirty-six hours, with the lesions of experimental diphtheria. The bacillus was recovered from the seat of inoculation, but from none of the organs.

CASE XV. From a lung in the stage of gray hepatization of a lobar pneumonia. Annie K., aged fifty-eight years. Autopsy by Dr. Wright.

At the autopsy portions of both lungs were found to be consolidated, and were in the stage of gray hepatization. One lung had partly broken down, and a thoracentesis had been performed. The culture from a consolidated area in one of the lungs developed Klebs-Löffler bacilli (not numerous) in addition to numerous colonies of streptococci and a few of the staphylococcus pyogenes aureus, as well as other bacteria. The cultures from the blood, kidney, spleen and liver showed the presence of a streptococcus septicemia. The throat was not examined. A guinea-pig inoculated in the usual manner with the Klebs-Löffler bacillus died after about three weeks. No lesions found.

The organism agreed with the Klebs-Löffler bacillus in its morphology and in its growth in blood serum and in litmus bouillon, which latter was reddened. The production of acid thus indicated is said by Escherich, as before noted, to occur only with the virulent form of the organism. The bacillus has not proven virulent and constitutes another exception to that writer's statement.

CASE XVI. From the skin of the thigh of a diphtheria patient. Beatrice C., admitted to hospital with diphtheria. As the child lay in its crib with one thigh exposed, a culture was obtained by rubbing a swab over the skin where a dark reddish area was observed. This area of discoloration was evidently due to a puncture with a hypodermic syringe. There was no ulceration. A moderately large number of colonies developed, among which was a fair proportion of colonies of the Klebs-Löffler bacillus. This, when inoculated into a guinea-pig, caused its death in seven and a half days. The animal weighed 230 gms., and received .2 c. c. of a twenty-four-hour bouillon culture of the second generation. At the autopsy of the animal dense induration and exudation in the tissues about the point of inoculation were found, together with lymphadenitis and some edema of the lungs. The spleen was large and soft. The suprarenals normal. The bacillus was recovered from the seat of inoculation, but from nowhere else.

CASE XVII. Unsuspected diphtheria. Autopsy by Dr. Wentworth on the body of a child.

The child had been wearing a trecheotomy tube for some two months, in consequence of an operation on the larynx for a new growth. Death occurred rather suddenly after a slight rise of temperature lasting about three days. The organs were brought to the laboratory for examination. Diphtheritic membrane was found in the trachea about the bifurcation. No broncho-pneumonia. A culture from this showed the presence of Klebs-Löffler bacilli and streptococci. No animal inoculations were made.

The striking thing about the Klebs-Löffler bacilli

obtained in these cases is the large proportion of mildly virulent or non-virulent forms among them. Out of 12 cases in which the virulence has been tested, three have yielded bacilli of little or no pathogenic effect on the animals, and in three other cases the guinea-pigs inoculated from them have survived seven and a half days and longer. It may therefore be said that a mild degree of virulence, or none at all, has been observed in 50 per cent. of the organisms so obtained which have been tested.

These results are in accord with the experience of others, as noted in the first part of this section. Whether this lack of virulence of the bacillus is due to attenuation by long continued residence away from its normal habitation, the air-passages, future study may decide.

From a consideration of certain of the above cases, we think that we are justified in holding that any excoriated or ulcerated surface on the body of a diphtheria patient will usually be found to be infected with the bacillus of that disease.

V.

On the morphology and biology of the Klebs-Löffler bacillus.

The Klebs-Löffler bacillus in its morphology is the most characteristic and at the same time the most variable organism known to bacteriologists. As has best been shown by Abbott,^{24, 26} its morphology varies markedly with the medium upon which it is grown, so that the bacilli from a blood-serum culture will have little resemblance in form to their descendants growing on an agar culture.

The bacilli from bouillon cultures are also different in morphology from these. Its microscopic appearance, when taken from a blood-serum culture and stained with Löffler's methylene-blue, is so characteristic that the practised eye can identify the organism by this alone. The gross appearances of the same culture are also of importance, though less characteristic.

In our work we have based the recognition of the Klebs-Löffler bacillus mainly upon its macroscopic and microscopic peculiarities when growing on this medium and, in most cases, in bouillon as well.

After a large experience in the study of the morphology of the Klebs-Löffler bacillus under these conditions, we think that at least two forms of it may be recognized. One of these is the more common, and is distinguished by being a more or less segmented rod with intensely staining ends and perhaps one or two sharply defined intensely staining areas between. The other form is much longer and much more segmented, often somewhat constricted at the middle and gently swelling toward either end, one of which may be somewhat thicker than the other. This long form presents usually two slight curves. Aside from its greater length, it is also distinguished from the shorter form by not commonly presenting the sharply defined, deeply staining points in its protoplasm; but it is irregularly segmented, each segment staining fairly deeply and uniformly, and being separated from the next by a narrow, faintly-stained interval.

We have satisfied ourselves that these long forms are a distinct type of the bacillus, and are not the product of special conditions. They have occurred only in a small proportion of the cases. This form does not change in successive cultures.

The existence of the different forms of the Klebs-Löffler bacillus has been noted by others. Martin³⁵ recognizes three — a short, a medium and a long form, and claims that there is definite relation between the severity of the case and the form or type of bacillus found in it. In our cases, however, we have not made out any constant association of either one of the two forms alluded to, with either mild or severe cases of diphtheria. Moreover, we have not observed any constant differences between these two forms as to their virulence towards guinea-pigs.

As to the much-mooted question of the pseudo-diphtheritic bacillus, we are of the opinion of Roux and Yersin,¹ Abbott⁶⁷ and others, that in many instances the organism to which this name has been applied is nothing but a non-virulent form of the Klebs-Löffler bacillus. The work of these investigators and others, as well as our own results, shows that there are Klebs-Löffler bacilli which are not virulent towards guinea-pigs, but which are otherwise identical with virulent bacilli. This is not surprising in view of what we know of the great variation of other bacteria in their effects upon animals.

These non-virulent forms of the diphtheria bacillus seem to have been called pseudo-diphtheria bacilli by some writers, owing to the apparently deeply rooted conviction that all true Klebs-Löffler bacilli are virulent towards guinea-pigs, which is not true. The term "pseudo" should no more be applied to a non-virulent diphtheria bacillus than to a non-virulent specimen of the frequently deadly staphylococcus pyogenes aureus.

In addition to these non-virulent forms of the true Klebs-Löffler bacillus, v. Hofman,⁵ Zarniko,⁶ Escherich² and others have described more or less clearly other non-virulent bacilli which resemble the Klebs-Löffler bacillus very much, and yet differ from it in certain respects. These they have also named "pseudo-diphtheria" bacilli. In the course of our studies on diphtheria and other pathological processes, we have not infrequently met with organisms of this character; but their morphological and cultural peculiarities sufficed after careful study to exclude them from consideration. We consider that these and the so-called pseudo-diphtheria bacilli of Escherich and others have nothing more to do with the causation of diphtheria than scores of other bacilli which may be found in the pharynx.

In conclusion, a point in the biology of the Klebs-Löffler bacillus deserves attention. As is well known, the diphtheria bacillus in its growth in neutral or slightly alkaline bouillon, produces an acid reaction in the medium. This has been regarded by some writers as one of the characteristics by which it may be distinguished from the so-called "pseudo-diphtheria" bacillus. Escherich,² moreover, considers that the production of the acid reaction, as shown by the changing of the violet color of the litmus-sugar bouillon to a red, is a reliable sign of virulence in the culture. He has recently stated that he has never seen an instance where an acid-forming culture has failed to kill the animal. This has not been our experience, for we have tested the acid production by the bacilli of most of our cases in the same way that Escherich did. We

have found that the bacilli from all sources which have shown little or no virulence, have all without exception turned the litmus bouillon red. These cases which have furnished organisms of little or no virulence have been 21 in number, in 20 of which the culture used for testing the acid production was also used for inoculation. We cannot, therefore, agree with Escherich as to the significance of a reddening of the litmus bouillon cultures.

Lastly, it may be said that we have never observed a satisfactory instance of a change of the red color of the bouillon back again to violet or blue in any of the large number of bouillon cultures which we have kept for a long time. This is surprising in view of the results of others, who have found that the acid reaction which first appears, later gives place to an alkaline reaction, so that we should expect to have the color of the litmus change accordingly.

All of the studies contained in this paper have been carried on under the direction of Professor Councilman of the Harvard Medical School, to whom the writer is under many obligations for good suggestions and active interest in the work.

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