Diphtheria.

Modern progress in its recognition and treatment.

This disease is remarkable as having shown a decided increase during the past few years, in spite of all modern improvements in sanitation, both as regards reconstruction of houses, the provision of a pure and plentiful water supply, and the disposal of sewage and refuse. Its distribution is also attended with remarkable and apparently inexplicable variations, which do not seem to be in any way dependent on meteorological or geological conditions.

As the disease is essentially one affecting young children, careful enquiry has been made by many observers into its chief means of conveyance of the conditions under which infection is most frequently transmitted. There is considerable evidence tending to show that schools play a prominent part in causing epidemics of this disease, children being brought together for several hours of the day, possibly
using the same slate pencils or transmitting the disease by kissing. So many observations have been recorded of the persistence of the bacillus in the throat for considerable periods, even amounting in many cases to seven or eight weeks after apparent complete recovery from the disease, that the necessity for a complete prolongation of a quarantine after convalescence, especially when attending school, should be insisted upon, not only this, but permission to return should only be accorded, when the final disappearance of the bacillus from the throat has been proved bacteriologically. In the same way many cases of sore throat occur which cannot clinically be recognized as true diphtheria, for which, nevertheless, the bacillus is present, is capable of infecting another host of giving rise to the disease in its full virulence. It is also noticed that prior to most epidemics of diphtheria, there have been a large number of ill defined sore throats of a quasi-diphtheritic kind.
Professor Smith in a recent report for the School Board of London (Journal of State Medicine May 1896) states, that in his opinion elementary schools do not play so great a part in the dissemination of diphtheria as is usually supposed, as he proves this point by the collection of a mass of statistics dealing with cases which have come under his notice as medical officer of the School Board. In no case, he says, has it been necessary to order the closure of the school on account of the outbreak of diphtheria. Accompanying his report are a series of most interesting coloured maps, illustrating the prevalence of diphtheria in each county during the past six years, which go to show that diphtheria has for some reason which we do not at present understand, become concentrated in the neighbourhood of Middlesex.

Dr. Hill, Medical officer of health, Birmingham, confirms Professor Smith's view, as he states that schools have very little to do with the spread of diphtheria so long as proper precautions
are taken to keep away all children from infected houses as is done in Birmingham.

The life of the organism is shortened by a few days by exposure to sunlight; it is also affected by desiccation. Hülle (Zeitschrift für Hygiene 1894) arrived at the conclusion that the diphtheria bacillus perished at that degree of dryness, which was necessary to permit the formation of dust. The accuracy of this statement is a matter of considerable importance, as it practically determines the extent to which the air can be regarded as a means of distributing the infection. The experiments of Reye (Annali d’Igiene 1895) however contradict this statement; working with virulent agar cultures, he suspended small portions in sterilized distilled water, in emulsions, cloth, sick, blotting paper, earth and sand; he exposed these in various experiments both in sunlight and in the dark, in both dry and wet. In some experiments the drying was conducted with
Sulphuric acid fumigation, as would occur in practice, was employed. In one case, with the object of directly examining Flügge’s conclusions, a quantity of infected powdered mud was placed in a sterile drying stove through which a current of air passed continually, watch glasses containing sterilized water being placed at various heights in the stove; all these glasses proved to be infected with the organism, which was of full virulence. The net outcome of this research, is to establish that the presence of moisture favours the growth of the organism, although it does not immediately perish on desiccation, thus when desiccated in the presence of sulphuric acid it may survive as long as forty-eight hours. When however it is desiccated in the ordinary way, it will remain alive in cloth, silk, or paper, for several days, in sand, for over two weeks, in powdered mud, up to one hundred days. The survival is still longer when
These media are moist, extended, in the case of sand, to more than one hundred and twenty days. Exposure to diffused sunlight only reduces the life of the organism by a few days; a temperature within ordinary atmospheric limits, exercises no appreciable difference. Without assuming that these results would be quantitatively true of all specimens of the diphtheria organism—an assumption which is care freely to be avoided in all references from bacteriological experiments—it is clear from these results that even after an amount of desiccation permitting the organism to be freely carried by the atmosphere, dust may contain living and virulent diphtheria bacilli, and the air must be regarded as a powerful means of spreading the disease. This result is in harmony with the generally received opinions; but it must not be taken to imply that air is the sole important agent for the natural distribution.
of the infection. There have been several researches, which have demonstrated the fact, that the diphtheria organism may be capable of growing freely in water.

In considering such experiments, it must be remembered that the term "water" is only a convenient expression for a solution, varying infinitely in its composition, containing in its natural state an infinite variety of bacterial life. It follows, therefore, that a negative result in endeavours to cultivate the organism in a given water may not necessarily apply in general terms to the same organisms over all waters; this reservation is the more important because a similar variation may exist in the capacity of the individual specimen or organism for growth in water. A positive result, however, if sufficiently free from disturbing factors, must be accepted as evidence of the capacity of the organism, in some conditions, to develop in water, as it is
unfortunately impossible to recognize the minute differences between various waters of various specimens of the same organisms, which tend to determine in the one case, the survival, in the other, the death of the organism, when inoculated into the water; a positive result obtained in trustworthy experiment must be held sufficient to show the liability of waters to carry the infection which the organism determines. The experiments of Dr. Vecchides (Arch de Med. Erb 1875) are the best evidence of this capacity in the diphtheria bacillus. He showed it in sterilized distilled water and found that it began to diminish only on the 7th day and not disappear until the 28th day. In such results the question always rises, whether the water was not rendered more favourable to the growth of the organisms by the accidental introduction or inoculation of minute quantities of the nutrient matter on which the organisms had been cultivated.
The experiments of Demetriades are probably not affected by this source of error, for on similar inoculations into spring water containing its natural quantity of dissolved organic matter, the diminution of the bacillus' life ultimate disappearance was delayed longer, than in the experiment with sterilized distilled water; showing that the relatively small proportion of nutrient matter ordinarily found in water was capable of determining a difference in the growth of the organisms, which was at least large relatively to the assistance afforded by particles of nutrient matter hypothetically introduced. For instance, with sterilized spring water, the diminution of the bacillus did not occur until the 19th day, pit survived till the 31st. With various unsterilized spring waters, the organisms survived multiplied still longer, for times y to extent which appeared to vary with the impurity of
the water, in particular, with the amount of dissolved organic matters which it contained. It is thus evident, that the
Bacillus thuringiensis has a capacity of growth in distilled water of that in unsterilized natural waters, its vitality in the presence of other micro-organisms is entirely exceptional in a non-aqueous microbe.

Taken as a whole, the experiments show that a bacillus may survive for some weeks in spring waters of little organic impurity, although during this time the virulence is gradually attenuated.

If, however, at any time previous to its ultimate disappearance, the organism be transplanted into a suitable culture medium it can reacquire its full initial virulence. Such a medium may be a human throat in a suitable condition of the research, therefore, establishes the fact that drinking water must be added to the
list of the means capable of spreading diphtheria.
Its importance in this respect is the greater in the light of the entirely independent results of Reyes, showing the
great capacity of the organism to survive in damp earth which
when once infected may for very long periods continue to
infect any water flowing through it.
Aber (Centralb. f. Bakf. May 4, 93)
on noticing that in winter diphtheria
often increased, made a series
of experiments on the action of
cold on the bacillus and came to
the conclusion, that the virulence
of the diphtheria bacillus was
in no way affected by cold,
such as is liable to occur
in natural conditions.
The spread of the disease by the
agency of milk is also a
matter deserving special
attention, particularly as
the establishment of creameries
in so many parts of the country
may prove to be factors of
great importance in the dissemination of the disease. At these creameries the milk of a very large number of cows (say 5 or 6 hundred) is received twice a day together in a large tank before passing through the separator. Thus, if the milk from one cow or one dairy contains the bacillus of diphtheria, it would be impossible to avoid the infection of the whole. The skim milk so obtained is at some factories condensed in tins, where the bacillus would probably be killed, but at others the milk is sold for human consumption as the organism multiplies in milk. At an enormous rate, it is easy to understand how epidemics of great magnitude might be caused. The only remedy for this would be to insist on the sterilization of all milk, leaving the dairy factory or the constant supervision of inspection.
of all cows & employees. An epedeme of diphtheria due to the milk supply will exhibit features similar to those of a typhoid epidemic produced in the same way:

1st. the outbreak is sudden & many attacks occur together.

2nd. the greater proportion of households attacked will have a common milk supply.

3rd. the incidence of the disease will fall chiefly on the principal consumers.

Considering however the comparatively recent date at which diphtheria has become a disease, which the ordinary practitioner expects to find of the great increase in the means for accurate diagnosis, which the discovery of the diphtheria bacillus has provided, it is possible that the increase in diphtheria may be more apparent than real. That, at least to a large extent, it may be explained by the increased accuracy of diagnosis.
The importance of early recognition of the disease both for excluding a child from school or a milkman from his work cannot be exaggerated. In this paper I propose to deal more particularly with the bacteriological diagnosis of diphtheria, how it can best be taken advantage of by the general practitioner. Before dealing with the actual details respecting the obtaining of specimens of mucus, it will be well to briefly review the general characters of the organism. The bacilli of diphtheria were first described in 1875 by Klebs, but it was not until 9 years later that cultures were obtained by Löffler of the pathogenicity of the organism generally admitted. The organism grows readily on all media, with the exception of potato, it does not liquefy gelatine, is non-motile, grows best in the presence of air.
For the purposes of bacteriological examination, it is of course important to employ that medium on which the most rapid and characteristic growth takes place.

The addition of grape sugar or glycerine to ordinary agar is an advantage in promoting the rapid growth of the organism, but the blood serum medium of Löffler is undoubtedly the best. This is prepared by adding equal volumes of blood serum and broth to a sufficient quantity of grape sugar to produce a mixture containing 8% of sugar.

In the preparation of this medium, any kind of blood may be used, but if it is desired to get the best results as regards color and cleanness of the medium, the blood of horses should be employed. The principal point is this; the blood after being drawn from the animal must be left absolutely at rest in a covered jar kept standing in ice for eighteen hours until the clot has entirely separated, next day the clear
Serum can be drawn off by a pipette or syphon; any attempt to convey the blood to the laboratory on the same day that it is drawn from the animal will entirely prevent proper separation of the clot from the serum; it will be impossible to prepare satisfactory media therefrom.

It has been proposed by some to utilize any quantities of anti-toxic serum left over for the manufacture of media. If this is done some glucose should be added. It is hardly necessary to say that in the case of all media containing blood serum the tubes must be sealed when they are sterilized as they set at a temperature of about 50°C.

Joos (Journal de Bruxelles May 4, 1896) claims to have invented a material on which no other bacilli, but that of diphtheria, will grow normally. He prepares “albuminate of soda” by adding saturated caustic soda solution to serum of strong alkalinity.
placing the mixture in a vapor bath for an hour, filtering. The filtrate is added, pure hydrochloric acid till the reaction is neutral or very slightly alkaline; if too much caustic soda is present the excess requires to be dialysed. On evaporation to dryness a powder is obtained which is readily soluble in water which is not coagulated by heat. The nutritive medium is prepared by adding 1,000 g. of peptonized bouillon 20 g. each of agar-agar of "albuminate of soda". The mixture is placed in the autoclave at a temperature of 115° C. to 120° C for half an hour, then 15 c.c. of caustic soda are added of the whole put back in the autoclave for a quarter of an hour, after which it is filtered in the steam bath, after filtration it is sterilized at 120° C. in the autoclave for three quarters of an hour, after which it is ready for the preparation of plates. Igoe claims for the medium
that upon it Staphylococci do not
grow at all & Staphylococci but
feebly, while Löffler's bacillus
grows abundantly in 6 to
12 hours.

The ordinary outfit supplied by
Laboratories to medical men
for the purpose of the bacteriological
diagnosis of diphtheria, consists
of two stout glass test tubes
about 4 inches long & 1/2 inch
in diameter, both plugged with
cotton wool sterilized, one
containing medium duly
sloped, the other the inoculating
wire. An iron wire is frequently
used covered with cotton at
the end. The suspected portion
of the throat is rubbed with
the covered end of this wire and
then gently rubbed on the
surface of the medium in
the other tube. The cotton wool
plug is replaced in the tube
which is labelled with the
patient's name, age, 
& the address
to which the report is to be sent;
returned to the box & posted to
the laboratory.
In cases where an incubator is not at hand, if it is not desired to send a specimen to a laboratory, some observers have incubated the tube by placing it under a sitting hen. I have found, however, that it is quite satisfactory to incubate the tube on the person, which may be done quite safely if stout glass tubes are employed. I effected this first by slipping an old tie, placing the tube therein, fastening the tie round my waist, keeping it so for twelve hours.

As this is somewhat inconvenient, I also tried placing the tube in the waistcoat pocket during the day, buttoning the coat over it and found in every case that satisfactory results were obtained.

It is important to obtain, if possible, a pure culture of the Klebs-Löffler bacillus, if present; when a cotton covered iron wire is used this is not easy as too much of
the infecting matter will be spread on the surface of the medium. Some observers recommend the use of a goat’s hair brush, but this is also open to the same objection. I much prefer to use an ordinary platinum inoculating wire, which can be sterilized in the ordinary way used again and again. With this three streaks should be made on the medium, after touching the throat, once only. If this is done, probably a portion of one of these streaks will be a pure culture of the Klebs-Löffler bacillus, even if there are a number of contaminating organisms present. I have not found that the naked eye appearance of the cultures can be relied upon in any way, as there are several common organisms, Staphylococci, etc., which produce cultures extremely similar in appearance. The diphtheria bacillus, generally,
but not always, produces a number of colonies, which are close
to, but not in contact with the
main streak.
The cultures are generally sufficiently
grown in twelve hours of cover
glass preparations may then be
made. The most convenient
stain to use is Löffler's methylene
blue, but the bacilli may be
stained by all the usual
aqueous basic aniline dyes
by Grams method.
Klein has described a pseudo-diphtheria
bacillus, which might be
mistaken for the Klebs-Löffler,
but it appears to be so rarely
met with (Newell found it
only twice in 1000 cases)
that for ordinary purposes,
may be disregarded.
The most reliable results of the
examination of a large number
of suspected cases of diphtheria,
are probably those published
by Sir Woodhead for the Metropolitan
Asylums Board of London. (Report
by S. J. Sims Woodhead. Upon the
bacteriological examination
of cases of diphtheria during the eight
months ended on the 31st Aug 1895.
In this report Dr. Woodhead
made Examinations of
2,901 cases taken from seven
hospitals under the M.A.B.
He 2,055 of these or 70.83 per
cent he detected the bacillus of
diphtheria and in 93.13 per
cent the bacteriological diagnosis
agreed with the diagnosis
entered on the bed card.
Also those of Dr. Hewlett Holan
of the British Institute of
Preventive Medicine (British Medical
Journal Feb 1st 1896). They comprise
the results of the Examination
of 1000 tubes of Löffler’s blood
serum, inoculated from suspected
cases, 41 of these nearly 59% yielded
the diphtheria bacillus; in the
remaining 958 the diphtheria
bacillus was absent. In 25
cases no growth was obtained.
They kept a record of 600 cases
as to the presence of other
organisms in the cultures; in 216 cases the Klebs Löffler
was present alone, in the
remainder in which it was found, it was associated with other organisms, which were chiefly micrococci. There is considerable body of evidence to show that the intensity of the infection may be aggravated by the presence of other organisms, as the streptococcus, less often the pneumococcus, is still more rarely the staphylococcus.

De Blasi and Russo-Travali (Annals de l'Institut Pasteur) have found considerable increase in virulence in few cases where the bacillus coli was present. Investigating this subject, experimentally on guinea pigs, they obtained the same result, which is important, owing to the ubiquity of the organism. Probably lends force to the direction of using antiseptics locally as much as possible. Dr. Hewlett-Yolow draw attention to the possibility that the bacillus may have been absent in some of the cultures, owing to imperfect rubbing of the
infected portion of the throat previous to the inoculation of the medium, there is another cause, which in some cases may have occasioned its absence, viz.: the use of antiseptic washes in the throat previous to the inoculation.

Klein in the Local Government Report 1893 records similar cases where specimens of the ileum, in cases of Cholera, were sent to him for examination, antiseptics having been added, thus rendering cultural tests impossible.

The table on the following page shows the result of 11 cases examined by me.
<table>
<thead>
<tr>
<th>Case</th>
<th>Klebs-Löffler Bacillus</th>
<th>Other Organisms</th>
<th>Subsequent History</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Present</td>
<td>None</td>
<td>Diphtheria</td>
</tr>
<tr>
<td>B</td>
<td>Present</td>
<td>Streptococci</td>
<td>Recovered</td>
</tr>
<tr>
<td>C</td>
<td>Absent</td>
<td>Staphylococci</td>
<td>Died</td>
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<tr>
<td>D</td>
<td>Present</td>
<td>Staphylococci</td>
<td>Recovered</td>
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<tr>
<td>E</td>
<td>Present</td>
<td>Coeci in Lungs</td>
<td>Diphtheria</td>
</tr>
<tr>
<td>F</td>
<td>Absent</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Present</td>
<td>Coeci Phlegma</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>Absent</td>
<td>None</td>
<td>Recovered</td>
</tr>
<tr>
<td>J</td>
<td>Present</td>
<td>None</td>
<td>Died</td>
</tr>
<tr>
<td>K</td>
<td>Present</td>
<td>Staphylococci</td>
<td></td>
</tr>
</tbody>
</table>

The text at the bottom of the page is not legible.
The bacillus has been traced in the throats not only of convalescents but also of those in attendance on diphtheria patients; it is therefore advisable to regularly examine the throats of nurses or attendants in diphtheria wards. A similar occurrence of a pathogenic organism in the throat of a healthy person is the discovery of the micrococci pneumonici in healthy sputum of the bacillus of typhoid has been found by Chauvemesse in the stools of a male nurse in perfect health who attended typhoid patients. I mention these cases with a view of showing that we must not in any way conclude that the finding of the klebs loffler bacilli in the throat of healthy subjects, is any argument at all against the pathogenicity of the organism of the bacillus; so present, may transmit the disease to other persons without having taken
Effect on the intermediate carrier of the bacilli cannot be demonstrated; we must not therefore conclude that the case cannot be diphtheria, as, where very few bacilli were present as at the commencement, it might be missed. In any case that presents the clinical symptoms of diphtheria, it should be treated as such, irrespective of negative bacteriological results. On the other hand we may often recognize a case of true diphtheria in which the clinical symptoms were so mild as to be scarcely recognizable, which though unimportant in itself, give rise to severe cases.

The main points of distinction between the diphtheritic membrane of the exudation of follicular tonsillitis are as follows. The former is deeply seated in the mucous membrane and not easily detached without
haemorrhage, while the latter is superficial and readily removed. The diphtheritic membrane is tough, having a consistency which may be compared to that of wash leather, it is not limited to the tonsils, but may extend over the mucous membrane of the pharynx and down to windpipe, downwards into the nose. It continues, unless arrested by treatment, for a week or more.

In contrast to this, the membrane in follicular tonsillitis, is more friable in texture, easily broken by the nails, it is limited to the tonsillic portion of the pharynx is generally of shorter duration as it ceases to appear even without treatment after 2 or 3 days. As in diphtheria considerable rise in temperature may occur. During Convalescence the bacillus of diphtheria, if present in the throat, will
most probably be found existing as long and short rods together, whereas at the commencement of the case it will be found in the form of short rods only; these short rods are usually 2 microns in length and 1/2 a micron thick, but after sub culturing they appear to lengthen and grow in size very considerably, particularly if blood serum is used, so that they appear to be quite double the size they were originally. Old cultures often take the stain unevenly as tho' shores were present. The short rods are generally, but not always, slightly clubbed at the end.

Attenuated varieties of the diphtheria bacillus have been obtained by Rouse, Gersic, and others, by cultivating it at a temperature slightly above blood heat and freely supplied with air, if they have suggested that a similar attenuation of
Pathogenic power may occur in the fauces of convalescents from the disease; that possibly the bacilli of low pathogenic power, which have been described by various observers may have, in this way, been produced from the true diphtheria bacillus. They also found that cultures of diphtheria obtained from false membrane, which had been kept by them in a dry condition, for some months grew readily but were destitute of virulence. In view of the fact that the life of the organism is not destroyed by drying, it is important in treating a case of diphtheria to provide, as far as possible, for the destruction of the organisms given off from the throat or nasal discharges. I have experimented as to the action of the following reagents on the diphtheria bacillus in broth culture 24 hours old with the following results...
The experiments were performed thus: Several small flasks were charged with 2 c.c. of a sterile broth culture inoculated with a pure culture of diphtheria overnight. When a 24-hour growth had been obtained 1 c.c. of each of the following solutions were added. Namely: 1/20, 1/50, 1/100. 1/500, 1/1000. Carbolic. Permanganate. 1/100, 1/200, 1/500, 1/1000, 1/2000. Mercurochloride.

This will produce a strength of 1/10 of each of the above solutions. The mixture of bacterial growth and disinfectant was left to act for precisely 10 minutes, then an inoculation made with a sterile platinum wire on to gelatin tubes. The results are best expressed in the following table:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>+</td>
</tr>
<tr>
<td>1/20</td>
<td>-</td>
</tr>
<tr>
<td>1/50</td>
<td>-</td>
</tr>
<tr>
<td>1/100</td>
<td>-</td>
</tr>
<tr>
<td>1/500</td>
<td>+</td>
</tr>
<tr>
<td>1/1000</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Permanganate</td>
</tr>
<tr>
<td>------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Control</td>
<td>+</td>
</tr>
<tr>
<td>1 in 200</td>
<td>-</td>
</tr>
<tr>
<td>1 in 500</td>
<td>-</td>
</tr>
<tr>
<td>1 in 1,000</td>
<td>+</td>
</tr>
<tr>
<td>1 in 5,000</td>
<td>+</td>
</tr>
<tr>
<td>1 in 10,000</td>
<td>+</td>
</tr>
<tr>
<td>1 in 20,000</td>
<td>+</td>
</tr>
</tbody>
</table>

From the above results it is clear that Mercuric Chloride is very suitable to destroy the organism in all cases where there is no objection to its use; this chemical is, however, hardly admissible for the purpose of circumscribing the growth in the throat, not only on account of its toxic character, but also by reason of its liability to decomposition in the presence of albumen.
it is of importance to circumscribe its growth from the outset by every possible means.

Löffler (Deutsch Med. Wochenschrift Oct 18th 1894) found that many solutions, ordinarily regarded as merely antiseptic, killed the diphtheria bacilli in a few seconds.

He favoured, in particular, the use of alcohol 60 parts, toluol 36 parts, 3% lye, and rubblic 4 parts, which he found killed fully developed diphtheria cultures in five seconds.

I am of opinion that experiments should be made with a view of ascertaining what is the best antiseptic than can be used for the early circumscribing of the growth of the diphtheria bacillus, as the more the growth of the organism can be prohibited from spreading in the throat, the less fear there will be for the necessity of performing tracheotomy, and the less toxcine will be produced. It is to this toxcine that the nerve paralyses are ascribed, which constitute
The worst sequelae in this disease hence, no pains must be spared in washing or spraying the throat with antiseptic solution. The discoverers of the diphtheria virus were inclined to refer the poisonous substances to the class of enzymes and to regard them as secretion products of the bacterial cells. Gamaleia, on the other hand, taking note of the fact that in spite of vigorous growth of bacteria in cultures, no poison could be found in them in the earlier days, felt the poison first taken to occur after a considerable time, when the multiplication of bacteria was reduced and the broth had become alkaline. He was inclined to regard the poison as contained in the bacterial tissues of extracted from by maceration in the alkaline fluid. Kössel (Centralblatt für Bak. 19, 25) determined that in suitable conditions the poison
Passes into the broth from the beginning of multiplication is therefore not due to a mere infection of the dead bacteria, which attain much greater numbers at a later period when the production of poison is reduced.

Guinochet (Archives de Médecine Experimenterale IV 1894) and Ouschkinsky (loc. cit. V. 1893) found that the poison could be obtained by cultivating the organisms in a media free from albumen and concluded therefore that it was developed by a synthetic process in the cells themselves.

Frankel (Le Rundsch 1894. 17.) recently Hugonnencq and Boyer (La Proceen Medicine 1896. 19) have, however, failed to get any growth to speak of on media such as Ouschkinsky describes; and Kossel (loc. cit.) by repeatedly centrifuging a broth culture, so as to separate the broth from

...
The bacterial portion, then killing the bacteria with chloroform,
extracting them with a little weak alkali fluid; found that the bacterial
tissue did contain poison, but, in far too slight a measure, to account for
the poisonous nature of old cultures. He therefore arrived at the conclusion,
which probably may be accepted as correct, that the production of poison
is due to a process occurring in the bacterial cells or a
secretion which arises therefrom.

Antitoxin treatment of diphtheria. The antitoxin treatment of
diphtheria has now received a
fair trial at the hands of
experimenters in almost every
civilized country; in no case
where it has been applied to a
considerable number of patients
have results been reported which,
on the whole, compare unfavourably
with any other method of
treatment with which we are acquainted. The comparison of statistics obtained by different observers is generally a matter of difficulty, because care has not been taken to note the age of the patients, whether or not antiseptic washes were used for the throat, nor is information usually given of previous statistics before the introduction of the treatment. Isolated cases are of course useless as a means of forming an opinion as to the success of the treatment. In spite of the difficulties introduced by our present inability to form an exact estimate of the amount of dose that will be most beneficial, distinct advantages are usually obtained by its use. At the present time the treatment is far more suited for use in institutions where a large number of diphtheritic cases are received, & a
Systematic bacteriological examination is carried out, rather than in private practice. It is found that the best results are obtained when the treatment is adopted as early as possible. In mild or very severe cases, but little advantage occurs. In any case, care must be taken not to preserve antitoxin serum which has been opened for fear of blood poisoning. The greatest care must of course be taken to ensure sterility of the injecting syringe. With the greatest care it is impossible to avoid the appearance of various rashes, which are occasionally similar in appearance to those of scarlet fever or measles, or more frequently erythematous or urticarial. The usual time for the appearance of these rashes is about the eight or ninth day after the injection.
Some people have alleged, that the injection of serum has actually caused nephritis, but this is contrary to the experience of those who have had an extended experience of serotherapy.

Preparation of diphtheria antitoxin. There are now no less than four laboratories in this country, which undertake the preparations of this remedy, namely:

1. The Laboratory of the Corjoint Board, The British Institute of Preventive Medicine.
2. The Leicester Institute of Health.
4. The method employed varies slightly at each institution, but the following is the general method employed:

A virulent culture of the diphtheria bacillus is taken grown in broth to which grape sugar has been added, in flat bottomed flasks,
Supplied with a regulated current of sterile air, at the temperature of 37°C, the liquid becomes cloudy after a few hours, but the growth is continued for nearly three weeks, when a sediment is found at the bottom of the flask, while the liquid has become almost clear again. The broth is then neutral, whereas, at the beginning of the operation, the broth was of course slightly alkaline; the liquid is then filtered through a Pasteur-Chamberland filter, if the amount of toxin produced is estimated by injecting it into a guinea pig. The ordinary standard of virulence is this, viz., that 1/10 of a centimetre shall kill a guinea pig weighing 500 grammes in twenty-four hours. It is then ready to start the immunisation of an animal, horses are used for this purpose because they are able.
to yield considerable quantities of serum without injury to their health. A small quantity, say 10 centimetres, is injected into a horse which is, as far as can be ascertained, in perfect health. A slight swelling will appear at the point of injection, but will subside again after a few days, when the operation is repeated, a larger quantity of toxin being used. After a further lapse of a few days a third injection is made, and so on until the animal can bear the injection of a large quantity, say 200 centimetres, without disturbance to its health. It is now ready to yield antitoxic serum which is obtained in the following manner: The animal is bled with antiseptic precautions of the serum preserved for use; the pain and inconvenience caused to the animal must be exceeding small as the horses go on quietly feeding during
the operations. A sterile cannula is placed in the jugular and the required quantity of blood drawn off into sterile bottles. When the clot has separated, the clear serum is put up in small stoppered bottles, or sealed up in tubes, or it may be evaporated and sent out in the form of scales which are soluble in 3 or 4 parts of water. Shwack (Ann. de l'Inst. Pasteur 18, 10) found that the necessity for boiling the tissue when preparing it in flat-bottomed bottles was avoided by using meat in which decomposition was just beginning; he attributes this result to the absence of glucose in such meat. On this method the broth would be made preferably from beef kept until decomposition began to occur; 2 per cent of beef t-bone free from glucose would be added. The solution after being carefully rendered alkaline would receive 5 per cent of chloride of sodium.
Y a small quantity of Carbonate of lime.

Nicolle (loc. cit. x. 6) found better results than he was able to obtain from decomposing meat, by macerating in twice their weight of water pieces of beef killed the same morning, adding 2 per cent of peptone and 5 per cent of salt, boiling, filtering, and rendering strongly alkaline, then heating for 10 minutes at 120° C., filtering again and sterilizing in the ultimate flasks of any form in which the mixture was prepared. Inoculation should be made from a young culture.

Which of these methods is in fact the better, depends on various undetermined conditions connected with the different species of organisms and composition of the materials used in the culture media. Nicolles experiments are conclusive against Shipock's method, having been as satisfactory to him as his own and also
against the advantage to his own work of the conclusion of decomposition which Spronck in his experiments found essential. On the other hand, Kossel in his experiments (Central für Bakt. 19, 25) found the use of meat approaching to decomposition to have the advantages which Spronck indicated. The exposure of toxin to sunlight again exercises a marked effect on its virulence, as has been clearly shown by Roux, Berzin and Piazza, the attenuation amounting to as much as 1/2, after direct exposure to sunlight for only two hours. The use of antitoxin serum in this country has not been attended with such success as abroad, yet it is probable that this is due partly to the weak immunising
power of the serums supplied in this country.
A most valuable report was issued by a Special
Commission appointed by the
Lancet, printed in that
journal 18th July 1896.
Any person who proposes to
employ Antitoxic Serum
should study it, as it affords
practically the only information
we have of the true value
of the serums made in this
country.
In this report all the serums
in use in this country,
including some made abroad
were examined and reported on.
The conclusions arrived at
were briefly as follows:
(1) That a common standard of
estimating the strength of
antitoxic serum should be
agreed on by the English
manufacturers.
(2) That no serum should ever
be sent out containing less
than 60 normal antitoxic
units per c.c.
(3) That antitoxic serum of higher strength must also be provided to meet the requirements of treatment in more severe cases of diphtheria.

(4) That every sample of antitoxic serum sold should be plainly marked with the antitoxic strength of the serum (number of normal antitoxic units per cc.), the quantity of serum present in the bottle, and the date of issue.

It must be taken, in fact, that the experience at present available in the antitoxic treatment, relates to the use of antitoxins, which, by the operation of the above factors, probably by many others, are essentially different one from the other, and the present time we see that the most pressing need for the scientific application of serotherapy is some accepted standard of strength for antitoxic sera, and the consequent
development of results which may be taken as more strictly comparable than those at present available.