The anti-toxin treatment of diphtheria / by John Glaister.

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The Anti-toxin Treatment of Diphtheria.

By JOHN GLAISTER, M.D., D.P.H.(Camb.), &c., Professor of Forensic Medicine and Public Health, Saint Mungo's College, Glasgow.

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THERE is probably no department of the science of medicine which has made more rapid and enormous advances during the last twenty-five years than that which has been named Preventive Medicine. This branch may be sub-divided into several sub-branches, all of which, working on different lines, and in different fields, have one common characteristic—namely, the discovery of the more occult processes of nature, whereby the health of man is affected.

While much has been done by general hygiene to remove and modify many factors which operate prejudicially upon the health of people in the mass, much has also been done in one special branch of preventive medicine—namely, in the bacteriological,—in the pursuit of those causes which produce infectious diseases, which pursuit has been rendered more successful by the use of the microscope in its higher powers, and by the use of culture fluids in which the different micro-organisms may be sown, grown, and multiplied.

Microbes—literally, small living things—are to be found everywhere in nature—in air, earth, water, within and without our bodies. They operate in curious ways and in divers places, conservatively and destructively, and are both the friend and the foe of man. They rid the earth of dead and decaying matter; they form new chemical compounds in the course of these operations; in their grosser and fungoid forms, they produce the alcoholic fermentation in suitable liquids; they make our sweet milk turn sour, our butter to become rancid, our bread to become mouldy; and perhaps the most serious action which they produce is to be found in a large number of the diseases of man and animals, while, in addition, they are the prime movers in the causation of all

zymotic or infectious diseases of animals—man included,—in which connection they have been called microzymes, or small ferments, from their supposed mode of action.

A knowledge of them is of importance, alike to the agriculturist, the horticulturist, the physician, the surgeon, and the hygienist;—to the two first, in respect of their being the cause of many of our crop-blights, our potato-disease, vine-disease, and the various infectious diseases of animals; to the physician, in being the cause of many obscure diseases as well as the whole range of zymotic diseases; to the surgeon, in respect of their being the cause of putridity in wounds and of septicæmia; and to the hygienist, in respect that a knowledge of them and their life-histories is contributory to success in their prevention or the amelioration of their evil effects.

The evolution of time has brought about the closer study of those micro-organisms, concerning the very existence of which so much controversy has raged within the last twenty-five years, with the result that their existence is not only incontestably proved, but their isolation, their culture, and their actions, have become historical achievements. It is too late in the day to doubt or deny their presence; it is still open, however, regarding several of them, to speculate as to their action.

This Society, by its Sanitary Section, has always been keenly alive to the advancements of Preventive Medicine. One of its former Presidents—the late Dr. Andrew Fergus—did much in his papers to instruct the members and the general public regarding the evil effects of sewer-air and sewer-gas. Probably their effects were then over-estimated, and the effect of the study of their influences on health, which he did much to initiate, has been to relegate them to better-defined lines of action. This paper is an attempt to follow on the lines of the policy of the Society.

The attention which has been given of late years to the study of Preventive Medicine is already bearing fruit; every decade points to lowered general death-rates in populous communities; some diseases are well-nigh extinct; people are enabled to live more comfortably and to live longer. But after this has been said, much remains to be done. Although typhus is now a rare disease in Glasgow, where it once was a plague, enteric fever and other scourges still remain. These so-called preventable diseases are, in many cases, still unprevented, although it is undoubted that, year by year, we are realising the query of the Prince of Wales in his

opening remarks at the Hygiene Congress in London: "*Then, if preventable, why not prevented?*"

This leads me to say, briefly, what has been done in recent times in this regard, chiefly however, by Continental workers. Pasteur, by his researches into the cause of Anthrax—a disease of cattle and sheep,—has not only been able to track the bacillus or microbe of the disease, but he has also been able to produce a vaccine, which, injected into the bodies of unaffected animals, prevents the disease doing them harm. From this discovery alone, hundreds of thousands of pounds have been saved to the agriculturists and to the nation of France. His researches on Rabies are now known over the world, and even from Glasgow we are at this time of day compelled to send persons bitten by rabid animals to his Institute at Paris for treatment. Here, curiously enough, although the microbe of the disease has not yet been isolated, Pasteur, in 1884, was able to produce a successful form of treatment against the operation of the disease in the person affected. So, likewise, from the study of Glanders in horses—a disease which is communicable to man, and which is often fatal,—not only has the bacillus been discovered, but its active principle has now been isolated, and is being used to-day in the diagnosis of obscure cases of the disease—that is to say, by injecting into the body of a horse some of this *Mallein*, an answer can be definitely given as to whether or not the animal suffers from the disease. Thus, a diagnostic power of great value has been placed in the hands of the veterinarian. So also, from the study of Tuberculosis by Koch, we have obtained *Tuberculin*, which, although it proved a disastrous failure as a curative agent in consumption, is proving of great value in the diagnosis of the latent tuberculosis of cattle.

In addition to these, patient and painstaking research has resulted in the discovery of the microbe of Cholera by Koch, of the microbe of Enteric Fever by Eberth and Klebs, of the micrococcus of Measles by Batés, in 1880; of the microbe of Pneumonia by Friedlander and Talamon, in 1882; of the microbes of Erysipelas, of Scarlet Fever, of Tetanus, of Leprosy, and of Influenza; and of the bacillus of Diphtheria by Klebs, in 1873, and afterwards confirmed by Lœffler.

We have here, then, abundant illustration of the magnificent work which has been, and still is being, performed in the department of bacteriology. Our concern, to night, however, is with the microbe of Diphtheria, a disease which is wide-spread, which

has been universally dreaded because of its great mortality, but to which recent investigation has apparently produced an *antidote.*

STEPS LEADING TO THE DISCOVERY OF THE *BACILLUS DIPHTHERIÆ.*

Laycock,* in Great Britain, was probably the first observer who associated Diphtheria with a micro-organism; but his idea of the character of this microbe was very wide of the mark. This was in 1858. On the other hand, in the Report of the Committee on Membranous Croup and Diphtheria, appointed by the Royal Medical and Chirurgical Society in 1879, to investigate the etiology of these diseases, we find the Committee reporting that, while they admitted that different micro-organisms were found at one and the same time in the false membrane of Diphtheria, they did not associate them as being the causative agents of the disease. Then Oertel in Germany, finding a streptococcus in the membrane very constantly, believed that *it* was the etiological factor. Klebs next discovered a bacillus in the throat, which he described, but failed to isolate. It was not, however, till Friedrich Lœffler set about the investigation of the disease that the bacillus which Klebs described was isolated and grown, and which is now proved to have the relation to the disease of cause and effect. During the investigation, however, besides this bacillus, Lœffler came upon this chain-forming microbe already spoken of, and at first he was not certain whether this form or the rod-like form, or both, were the causal factors. Experiments on the lines laid down by Koch quickly settled this question, and now we know the *bacillus diphtheriæ* as the Klebs-Lœffler bacillus.

In the isolation and cultivation of these microbes, it is of interest to know that one of the modes by which they may be differentiated is by their preference for, or susceptibility to, certain dye-stains. The *bacillus diphtheriæ* is very susceptible to the dye known as methylene blue, one of the aniline colours, made up in certain proportions with alcohol and solution of potash—namely, 30cc. of concentrated alcohol solution of methylene blue to 100cc. of (1 in 10,000 of water) solution of potash. The tissue supposed to contain these bacilli is placed for a few minutes in this solution, then placed in a half per cent. solution of acetic acid, and next dehydrated in alcohol, and immersed in cedar oil and mounted.

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* *Medical Times and Gazette*, May 29*th*, 1858.

Lœffler, too, found when making cultures of the bacillus that the nutrient jelly of Koch was not suitable for its growth, although quite suitable for the other form of microbe. He discovered that it grew best in *coagulated blood serum*, made up of three parts of the blood serum of the calf or lamb, and one part of veal solution, containing one per cent. of peptone, one per cent. of grape sugar, and a half per cent. of common salt.*

What are the characters of the *bacillus diphtheriæ*? Although they may vary slightly in length, the average is from a quarter to half the diameter of a red blood corpuscle—that is to say, from ·0015 to ·0035 of a millimetre, and they are either straight or slightly curved, and somewhat thickened at one extremity. They are non-motile. They require for their cultivation and growth a temperature above 68° Fah. (20° C.), and they are destroyed by exposure for half-an-hour to a temperature of 140° Fah. (60° C.). Their average life in the most suitable environment is about three months.

In his experiments to test their casual relationship to the disease, Lœffler discovered the very interesting fact, that all animals were not equally susceptible to their evil influence. The following animals, and in the following order, showed increasing susceptibility:—hens and pigeons, small birds, guinea-pigs. The last named were quickly affected, while rats and mice were quite insusceptible.

The next point of interest to make clear is, in what part of the body of the person affected with diphtheria are these bacilli found? Let me answer this by saying where they are not found, namely, in the blood of the person attacked. They are always to be found, however, in the patches of deposit or false membrane, as it is termed, which are to be seen in the throat or air-passages, grouped in masses. They are invariably situated near the surface of the false membrane, for they require air for their growth; they are aerobic. But what causes the constitutional symptoms of the disease, namely, the fever, the prostration, and the occasional after-coming paralysis? The bacilli during the act of growth, and as the direct result of that growth, in using up the nutrient material which they find in the fluids of the throat and its tissues, create

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* *Mittheilungen aus dem K. Gesundheitsamte*, Vol. II., Berlin, 1884. *Vide* also "Micro-parasites in Disease" (New Sydenham Society, 1886), pp. 448, *et seq.*

new chemical substances of a poisonous or toxic character, called albumoses or toxines. These toxines, so formed, are absorbed into the blood of the person, and there give rise to the above-mentioned grave symptoms. Hence it is that, while the manufacturers of the poison or toxine live practically on the surface of the body, the toxine itself is introduced into the blood by absorption. Obviously, then, to overcome the constitutional effects of this toxine is to cure the disease; and hence the treatment has been termed the "anti-toxin" treatment.

We have now reached this stage of discovery—namely, (1) that a bacillus, distinct in character and effect, is the causal factor in the production of the disease; (2) that this bacillus can be isolated from the deposit upon the throat of a diphtheritic patient; (3) that it can be artificially cultivated outside of the body; and (4) that this artificial culture, or even a part of it, when injected into the body of a healthy animal, will produce both the physical and the constitutional signs of diphtheria. But, it may be asked, how came about the discovery of the principle of the sero-therapeutic treatment? It had already been established by several observers that if the microbes of certain well-defined diseases—anthrax and others—were cultivated out of the body, and their original virulence weakened, and if such attenuated viri were injected into the bodies of sound animals, such animals would not become the subjects of the virulent form of the disease; that is to say, they had become protected; and the experience of the last hundred years in our own country has abundantly shown and proved the protecting influence which vaccinia imparts against smallpox.

In this connection various Continental observers had been doing splendid work. Babès, of Buda-Pesth, with Lepp, in July, 1889, had established this principle in respect of the treatment of hydrophobia,* and Behring and Kitasato had published in 1890 an article on the immunisation of animals against tetanus;† but the credit of the application of the principle to the treatment of diphtheria must be given to Professor Jaime Ferrán, of Barcelona, who, in a communication to a Spanish journal, of date April, 1890, pointed out and described a safe and practical method of immunising animals against fatal doses of diphtheritic poison, which he himself had successfully employed.

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* *Annales de l'Institut Pasteur*, July, 1889.

† *Deutsche Medicinische Wochenschrift*, No. 49, 1890.

From animals to man is but a short step; and to Behring, of Berlin, must be ascribed the honour of applying the principle to man. Before this, however, Roux and Yersin, of the Pasteur Institute, had been able to demonstrate that the bacillus of this disease was capable of evolving toxic material.

If I were to summarise the points which led up to this stage, I would put them thus:—

(1) Lœffler and Klebs discovered the bacillus, studied its life-history, and proved it to be the causal factor in the production of the disease;

(2) Roux and Yersin demonstrated that during its growth it manufactured a toxic substance which produced the constitutional disturbances in the person or animal affected; and

(3) Behring manufactured the anti-toxin.

As Roux himself has expressed it, "Behring, therefore, has completed and crowned the edifice."*

The terms "protection," "immunisation," and "immunity," having already in the course of this paper been more than once mentioned, it behoves that I should explain what they mean.

What is Immunity?

Necessarily, the answer to this question must be somewhat speculative, but the speculation is limited by the knowledge we possess of certain phenomena observed in cases of infectious diseases. In the first place, we know that if an individual is seized with an infectious disease and recovers from the illness, most usually (for there are certain odd exceptions even in this) a re-exposure to the infection of the same disease will not affect him. We then say of this individual that he is *protected* against a subsequent attack of the disease by reason of this first attack; or, in other words, he is *immune*. If we take the whole range of infectious diseases, we shall find that, in certain of them, the immunity conferred by an attack is more enduring—that is to say, it covers a much longer period of time—than in others. This is not relative to the particular individual only, but is in the nature of the contagium itself.

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* *British Medical Journal*, Vol. II, 1894, p. 931.

Knowing these facts, we have still to satisfy ourselves how this degree of resisting power is conferred on the individual. By what physiological process is it produced? And this is where the speculative element or theory comes in. We at least can say this, that the resistance is the sum of the effects of the introduction, growth, multiplication, and death of the living micro-organisms in the body during the currency of the illness, whether the illness be induced naturally or experimentally. But the intimate cause of the immunity or resistance we cannot tell. Various theories, however, have been propounded on this subject; among others, by Klebs, Pasteur, Chauveau, Wernich, Grawitz, Buchner, and Wolffberg, but I do not stop to discuss these. We do know that if we take the blood of an animal which has survived an attack of an infectious disease, it will prove antagonistic to the blood of another animal which is at the time suffering from the same disease; in other words, it exercises an antidotal or anti-toxic influence over the toxic blood of the second animal. So also, is it true that, *ab initio*, certain animals are more easily rendered immune than others.

Starting, then, from the known fact that the blood of an animal which has successfully passed through an attack of an infectious disease is antagonistic to the poison in the blood of an animal suffering from the same disease, it makes no difference whether the disease has been acquired naturally by, or has been conferred artificially upon, the animal. As has already been pointed out, the blood of the first animal is antidotal to the poison in the blood of the second; or, in other words, the one is anti-toxic to the other, and the material which has this anti-toxic character may be called anti-toxin.

How is the Anti-toxin of Diphtheria prepared?

There are two main methods employed in the preparation of the anti-toxin serum:—

(1) By injecting the toxines produced in the growth of the bacilli into the body of an animal in doses of gradually increasing strength; and

(2) By injecting attenuated bacilli—that is to say, bacilli of lessened virulency,—together with their toxines, into the body of an animal, in doses made gradually stronger

The aim and object of the treatment, in either case, is to accomplish the complete immunisation of the animal, so that its blood will be thoroughly antagonistic to the toxines circulating in the blood of an animal actually affected. The first method is that employed by Behring, and afterwards by Roux, Ruffer, and others; the second by Klein, in his recent investigations at the instance of the Local Government Board.

They deserve some detailed consideration. In the *Annales de l'Institut Pasteur* for September, 1894, Roux's method is described as follows:—

The bacilli taken from a typical case of diphtheria are cultivated in a suitable culture medium. This culture is inoculated into a sterilised alkaline beef-broth, containing a half-per-cent. solution of common salt, and two per cent. of peptone, in a flat-bottomed flask, corked with a two-holed indiarubber stopper, each hole of which carries a glass tube, one acting as an inlet, the other as an outlet, while air is being aspirated through the flask. Soon after the bacilli begin to grow in the beef-broth, which has been heated to a temperature of 37°·C (98·6 Fah.), a current of moist air is drawn through the flask and over its contents by means of the glass tubes, and a flocculent deposit begins to fall to the bottom of the fluid, and which continues to fall for some time. At the end of a month the clear supernatant fluid in the flask, which contains the toxine, is filtered through a Pasteur-Chamberland filter, which permits the fluid containing the toxine to pass through, but retains the bacilli. This toxine is now sufficiently strong that 0·1cc. (one-tenth) will kill a guinea-pig, weighing 500 grammes (about $1\frac{1}{10}$ lbs.), in 48 hours, and it is ready for use on the horse.

The horse is now preferred for the preparation of the anti-toxin—first, because it has but small original susceptibility to diphtheria; and, second, because it can yield large quantities of its blood without suffering inconvenience. In an earlier stage of the experimental inquiry, however, the blood or serum of goats was used. A healthy horse having been chosen, which has been proved to be clear of any taint of tuberculosis or glanders by the injection of tuberculin and mallein, it is ready for treatment.

Into the animal are injected, at first small, then progressively larger, doses of this toxine, the effects being observed in the swelling produced at the seat of puncture (Roux prefers the front of

the shoulder), and in the rise of the animal's temperature, which symptoms are allowed to subside before the next injection—the quantity of toxine used at first being 1cc., rising gradually until the animal can bear with impunity a dose of 200cc. The period of time covered before the animal is completely immune is about three months (72 days).

Let us pause a moment to note what has happened in the blood of the horse as a consequence of the foregoing treatment. In the first place, the toxine was produced in the flask as the effect of the growth of the living organism; this toxine has been injected in increasing doses into the animal's body until the point is reached when the animal is absolutely invulnerable to diphtheria. During this period each succeeding injection confers on the animal a degree of toleration of the toxine, and this degree of toleration rises as the doses increase in quantity, or, in other words, the blood-serum has attained the power of neutralising, has become antagonistic to, the toxine injected—that is to say, the blood is now anti-toxic to the toxine. To prove this is simple. If some of the serum from the blood of the animal be placed in a tube containing some of this original toxine, the combined liquid injected into the body of a susceptible guinea-pig would not produce any effect; the one has neutralised, or destroyed, the virulency of the other.

We have, however, only attained this important fact—namely, that the blood of this particular horse is, to a certain undefined degree, antagonistic or anti-toxic to the poison or toxine produced by the growth of the bacillus. Obviously, it will be of the utmost importance that its exact potency be known, in order that we may learn what quantity of it to inject into a human body. To obtain this knowledge the serum must be tested against toxines of known potency; as the chemist would say, it must be "standardised." To accomplish this, we again call the lower animals to our aid, and we choose the guinea-pig. The mode of "standardising" now adopted is that which is known as Ehrlich's method. This method is very exact. Taking a guinea-pig about 500 grammes in weight, it is first ascertained what quantity of toxine will prove fatal to the animal. This gives a definite point from which to begin. Then by mixing a definite amount of this toxine with definitely graduated quantities of the horse-serum procured as already described, the precise point of dilution at which the serum proves completely antagonistic to the toxine is discovered.

This is shown by the harmless effect produced by the injection of the mixed liquids into the guinea-pig.

Let me illustrate this point further. Suppose that ten times the dose of toxine necessary to kill a guinea-pig be mixed in one solution with one-third its quantity of the anti-toxin blood-serum; in a second solution, with the same amount of toxine, but with one-quarter its amount of serum; in a third with one-fifth; in a fourth with one-sixth; and in a fifth with one-tenth. It will be obvious that the poisonous or toxic effect of the original toxine will be modified by the anti-toxic horse-serum in each of the series of admixtures in direct ratio to the anti-toxic potency of the blood-serum. By how much the toxine is overpowered by the serum is shown after one injection of each of the series of solutions into the bodies of guinea-pigs, by the symptoms which are produced in the animals afterwards,—these symptoms being the inflammatory tumour at the seat of injection and the rise of temperature. By this means the point or degree of dilution (and hence the degree of potency of the serum) is reached at which the toxine is rendered inert—that is to say, the point at which the serum (or anti-toxin) has completely neutralised the toxine. It has been found that the serum of a horse treated as described has an anti-toxic potency from fifty to one hundred times greater than the serum of an untreated horse.

A quicker way, but one less exact, is to inject into the body of a guinea-pig ·01cc. of the horse-serum, and in 24 hours thereafter injecting 0·5cc. of a virulent culture of the living bacillus—a quantity which would prove fatal to an unprotected animal. Should the guinea-pig exhibit no evil effects, then at once it is known that the horse-serum is sufficiently powerful for use in the human subject.

The serum is obtained from the horse after treatment by tapping the jugular vein with a sterilised instrument. The blood is collected in sterilised glass jars, which are then either kept air-tight, or into which are placed small chunks of camphor. Very quickly the blood separates into two layers—the lower being the clot, and the upper the serum, which is of a pale straw colour. After the clot has firmed, the serum is drained off into other sterilised bottles, after which it is "standardised" in the manner already described.

The principal objection which has been offered to this mode of preparing the anti-toxin according to Roux's method is the long

period of time which it demands, and Klein has been the first to raise this objection.* But in stating it, he does not mean to imply that the serum prepared by Roux does not correspond to the value which Roux attaches to it; on the contrary, he believes it has all that value, but he further believes that it is prepared at a needless expenditure of time, and, it may be added, with needless expenditure of the vital energy of the horse. Neither of these objections is of prime importance so long as the preparation is not a matter of urgency, since the animal is being well cared for during the process.

Klein's argument, to put it in his own words, is as follows:—"Roux," he says, "introduces over and over again large amounts of pure diphtheria toxine into a horse which has already, by previous injections of the pure toxine, been rendered to a certain extent resistant against this toxine (it is because of this resistance that he finds it necessary to increase the dose of the toxine)—that is to say, a horse that has, by previous injections of diphtheria toxine, become more or less resistant, must possess a corresponding amount of anti-toxine in its blood. But, since the two substances—namely, toxine and anti-toxine—are antagonistic and neutralise one another, it follows that each successive injection of a large quantity of pure diphtheria toxine into a given horse must neutralise a proportionate amount of anti-toxine already formed and in the blood of the animal. This possibly explains," he concludes, "the extraordinarily long time which Roux's horses take before their blood is rendered sufficiently anti-toxic."

This consideration led Klein to adopt a different plan, by which he claims to be able to manufacture the anti-toxin in a much shorter time. This plan differs from that of Behring and Roux in that, whereas these experimenters use in their injections pure toxine only, Klein uses attenuated bacilli—that is to say, bacilli whose virulency is lessened by their age—plus the toxine. Beginning in this way, Klein proceeds to inject more virulent bacilli (minus their toxine, however) as the animal becomes acclimatised, until it becomes completely immunised, observing intervals to enable the animal to recover after each injection. From this mode of treatment Klein has obtained potent anti-toxin at the end of 23 to 26 days. When the animal is bled to supply serum, two or three fresh injections keep the anti-toxic value of the serum up to the

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* *British Medical Journal*, Vol. II., 1894, p. 1,393.

mark. The potency of this serum is such that *one* part of serum is capable of protecting 20,000 to 40,000 grammes body-weight of guinea-pig against not merely pure toxine, but against living bacilli and toxine combined. This serum has frequently been used with success, the dose being from $1\frac{1}{4}$ to 2 drachms (5 to 10cc.).

This is not the time nor the place to describe the operation required for the application of the treatment; neither is it proper that cases of the disease subjected to the treatment should be here described. But, in general, it may be said that the operation itself is neither difficult nor dangerous, nor is the amount of pain accompanying it more than that caused by the prick of a sharp needle; further, it may be added, that it has proved successful in the very limited number of cases in which I have used it; and if the figures which I have to put before you are trustworthy—and there are no apparent grounds for any other belief—it promises to enable us to overcome not a little of the fatality of the past. One other remark on this point which requires to be made, is that the serum ought to be used as soon as the disease is known to be diphtheria. Not a little harm has been done, in my opinion, by waiting until other remedies have failed, by which time certain pathological changes have taken place in the body of the patient which the serum could not be expected to overcome.

In Great Britain the honour of having been the first to prepare the anti-toxin after Roux's method falls to the British Institute of Preventive Medicine, the directors of which are Drs. Amand Ruffer and M'Fadyen. When in London towards the end of last October, in search of information on this and cognate subjects, Dr. J. B. Russell and myself visited the laboratories of that institution. The day of our visit is so far memorable in that we were informed that the first anti-toxin of the first horse treated was to be drawn from the animal that morning. We were in the laboratory when the jars containing the blood arrived from the stable, and thus, in a very indirect way, we assisted at the first step in the preparation of the serum in this country. From this first supply, after being "standardised," we each received a satisfactory quantity, which has been used by me upon occasions with success.

Let us turn aside for a moment to discuss the prevalence of diphtheria in this country. That this disease is on the increase is a noteworthy result of the study of its statistics. If we take England and Wales, and London (and I take the figures of Dr.

Seaton, Medical Officer of Health for Chelsea, given at the Budapest Congress of Hygiene), for different periods, but continuous, we see this clearly :—

TABLE I.—ENGLAND AND WALES.

Mean Annual Death-Rates from Diphtheria per million living, in England and Wales, and in London, for four periods of three years each :—

	1881-3.	1884-6.	1887-9.	1890-2.
England and Wales, - -	144	166	173	192
London, - - - - -	213	227	315	377

In London, during 1893, 13,694 cases of diphtheria were notified, of which 3,195 were fatal; mortality rate = 23·3 per cent.

TABLE II.—LONDON.

Number of Cases of Diphtheria and Membranous Croup notified in London from 1890 till 1893 inclusive (Allan, *The Journal of State Medicine*, Vol. II., No. 5, p. 262):—

Year.	Diphtheria.	Membranous Croup.	Total.
1890, - - - -	5,870 ...	550 ...	6,420
1891, - - - -	5,907 ...	565 ...	6,472
1892, - - - -	7,781 ...	554 ...	8,335
1893, - - - -	13,026 ...	668 ...	13,694

The statistics for this disease in London, from 1881 to 1893 inclusive, show that the death-rate per million living has risen from 172 in 1881 to 760 in 1893, and that the mortality from diseases of the throat, other than diphtheria, has fallen from 321 per million in 1881 to 120 in 1893.

TABLE III.—SCOTLAND.

Deaths from Diphtheria in 1890-1-2 :—

Year.	Total Deaths.	Croup.
1890, - - - - - - - - -	1,018 ...	744
1891, - - - - - - - - -	830 ...	620
1892, - - - - - - - - -	807 ...	540

In order to arrive at an approximate idea of the total number of diphtheria cases in Scotland in each of those years—those recovering and those dying combined,—I have taken the figures for Edinburgh and Glasgow for the same years, and on the average in both cities, where notification of cases obtains, I find that one case is fatal out of every four cases reported; hence if we multiply the above total deaths in Scotland by 4, we get approximately the total cases of diphtheria, thus :—

Year.	Total Deaths.			Approximate Total Cases.
1890, - - - - - - -	1,018	× 4	=	4,072
1891, - - - - - - -	830	× 4	=	3,320
1892, - - - - - - -	807	× 4	=	3,228

In addition to these figures, we have, under the heading of "Croup," doubtless hidden cases of diphtheria, which, however, we need not discuss further.

From the Report of the Board of Supervision for 1892-3, we learn that from 3,139,549 of the total population of Scotland—that is to say, 93·2 per cent.,—in which the system of notification of infectious diseases prevails, the number of cases of diphtheria and membranous croup notified was 2,655; in the case of the former disease, however, the death-rate was 25·6 per cent., and of the latter 43·1 per cent.; and for the year 1893-4, out of a population of 3,539,823—the estimated population of Scotland for that year being 4,025,647,—the number of cases notified of diphtheria and membranous croup was 3,523, the death-rate from the former being 25·8, and from the latter 55 per cent.

TABLE IV.—GLASGOW.

Return of Cases of Diphtheria and Membranous Croup from 1890 (the year of the introduction of the Notification Act) to 1894 inclusive:—

Year.	Cases.		Deaths.		Mortality per cent.
1890, - - - - -	581	...	137	...	23·58
1891, - - - - -	479	...	132	...	27·55
(Figures from "Greater Glasgow" follow.)					
1892, - - - - -	581	...	163	...	28·05
1893, - - - - -	827	...	208	...	25·15
1894, - - - - -	967	...	254	...	26·26

TABLE V.—EDINBURGH.

Return of Cases of Diphtheria from 1887 to 1894 inclusive:—

NOTE.—The Notification of Infectious Diseases came into force in Edinburgh in November, 1879.

Year.	Cases.		Deaths.		Mortality per cent.
1887, - - - - -	256	...	57	...	22·26
1888, - - - - -	255	...	65	...	25·49
1889, - - - - -	354	...	98	...	27·68
1890, - - - - -	361	...	85	...	23·54
1891, - - - - -	207	...	48	...	23·18
1892, - - - - -	203	...	42	...	26·9
1893, - - - - -	251	...	62	...	24·7
1894, - - - - -	362	...	86	...	23·75

Statistics of the Treatment of Diphtheria by the Anti-toxin Method.

We learn from the *British Medical Journal*, Vol. II., 1894, p. 545, that the earliest published cases of this treatment were recorded in the *Deutsche Medicinische Wochenschrift*, April 27th, 1893, by Behring and Kossel. They were 30 in number, of which 24, or 80 per cent., recovered. In April, 1894, Ehrlich, Kossel, and Wassermann published in the same journal the results of 220 unselected cases treated with the serum of goats rendered immune by giving them increasing doses of dead diphtheria bacilli (cultures). Of the 153 cases of this total in which surgical procedure was not required, the mortality was 23·6 per cent. Then, in July of the same year, and in the same journal, Weibgen, from Hahn's Clinic in Berlin, reported 65 cases; certain cases of this total required the operation of tracheotomy, of which 44 per cent. recovered; of the others, 72 per cent. recovered. It must be noted, however, that the type of disease was benign.

If we turn to the French figures, the results of the treatment seem more striking. Generally speaking, the mortality from diphtheria in the Parisian hospitals, according to Roux, had scarcely ever been, prior to the serum treatment, below 50 per cent.; since its use, the mortality has fallen to less than 24 per cent., all the cases being diagnosed bacteriologically. For instance, in the diphtheria wards of the Trousseau Hospital, for the four years before the commencement of this treatment, the mortality was 51·7 per cent. of the total cases. From 1st February to 24th July, 1894, the new treatment was used in 448 children, of whom 109 died—the mortality per cent. being 24·5. During the same time, at the Trousseau Hospital, the old lines of treatment were persevered in; of 520 total cases, 316 died—the mortality rate being 60 per cent. To show that, in the period of time chosen, the results were not attributable to a benign type of disease, Roux points out that, of the cases treated by serum at the Hospital for Children, the mortality fell to 12 per cent., while, during the same period, at the Trousseau Hospital, without serum, the mortality was 32 per cent.

Perhaps one of the most convincing statistical results that has yet been printed was that which was given by the veteran pathologist, Virchow, in a discussion on the merits of this treatment at the Berlin Medical Society. The discussion was opened

by Dr. Hansemann in a speech showing uncompromising opposition to the treatment. Virchow followed later, and, as a previous speaker had remarked while speaking that "a burnt child shuns the fire, and from tuberculin I had carried away bad burns," it was to be expected that Virchow's views would be characterised by considerable caution and conviction. He told his audience that the new treatment was begun in March, 1894, in one of the Berlin hospitals, and that, by June and July, all the diphtheria cases admitted were treated with the serum.

The results were as follow :—

Table VI.

Weeks.	Children. Cases Cured.	Deaths.
1	13	1
2	9	1
3	6	2
4	12	1
5	6	2
6	1	1
7	3	0
8	5	0

At this point the supply of serum suddenly ceased, because of the death of the horses which were supplying it; and the hospital staff were compelled to fall back on the old lines of treatment. This was the result :—

Weeks.	Cases Cured.	Deaths.
1	5	7
2	6	8
3	6	6
4	8	11
5	8	5
6	8	12
7	13	6

Alarmed at the increased mortality, the hospital authorities obtained a new supply of serum, and began to use it. The following was the effect :—

Weeks.	Cases Cured.	Deaths.
1	3	2
2	4	1
3	14	1
4	14	2
5	17	1
6	17	5

That is to say, slumping the figures together, the total number of cases was 533. Of these, 303, *treated with serum*, had a mortality of *13·2 per cent.*; while the remainder, *treated without serum*, had a mortality of *47·8 per cent.* After the narrative of these facts and figures, Virchow added that "all theoretical considerations must give way to the brute force of these figures"; and while he held it to be the duty of every physician to use this remedy in every case of diphtheria, he frankly owned that he could not explain its action. Prof. Baginsky, of the hospital in which these results were obtained, corroborated the accuracy of the foregoing statistical details, and added that "no previous remedy had done for diphtheria what the serum had done."

If we turn to our own country, we obtain evidence of the value of the treatment in the Annual Report of the British Institute of Preventive Medicine (*Lancet*, 2nd February, 1895, p. 305, *et seq.*)

Table VII.

Illustrating Treatment of Diphtheria at the Western Fever Hospital, London:—

Without Anti-toxin.

Cases admitted from November 26, 1893, to January 25, 1894—

Age Periods.	Admissions.	Deaths.	Percentages.
0 to 5 years,	20	12	60
5 to 10 ,,	21	6	28·57
10 to 15 ,,	7	—	—
Upwards,	10	1	10
Totals,	58	19	32·85

Table VIIa.—With Anti-toxin.

Cases admitted from November 26, 1894, to January 25, 1895—

			Type of Disease.			Complications.	
Age Periods.	Admissions.	Deaths.	Severe.	Moderate.	Mild.	Rashes.	Arthritis.
0 to 5 years,	40	5	15	18	7	10	1
5 to 10 ,,	22	5	13	7	2	6	1
10 to 15 ,,	4	—	2	2	—	3	2
Upwards,	2	—	2	—	—	1	—
Totals,	68	10	32	27	9	20	4

Death-rate = 14·7 per cent.

The foregoing cases were all certified by bacteriological diagnosis.

In the North-Western Fever Hospital, of 43 cases, diagnosed as before, treated with serum, only 2 were fatal—the mortality rate being 4·6 per cent. Of these cases, only 7 were deemed severe.

Again, in a joint-paper, read before the Clinical Society of London by Drs. Washbourn and Goodall and Mr. Card, the following statistics were given :—

Table VIII.

Case-Mortality of Diphtheria in Children under 15 years, at the Eastern Hospital, London—

Periods.	Cases.	Deaths.	Mortality per cent.
1893, - - - - - - - -	397	166	41·8
Jan. 1, 1894, to Oct. 22, 1894, - - -	400	144	36·0
Jan. 1, 1893, to Oct. 22, 1894, - - -	797	310	38·8
Sept. 14, 1894, to Oct. 22, 1894 (39 days), *not treated with Serum*, - - -	72	28	38·8
Oct. 23, 1894, to Nov. 27, 1894, *treated with Serum*, - - - - - -	72	14	19·4

—thus showing, in the two latter periods, from the treatment, that the mortality was reduced *50 per cent.*

The *British Medical Journal* opened its columns for the recording of cases treated in the new way, and between the beginning of November, 1894, and the beginning of February, 1895, there were 95 cases recorded by different observers: with 22 deaths—thus giving a percentage mortality of 23·1.

Table IX.

(Compiled from the *British Medical Journal*, February 2, 1895, p. 259.)

Total Cases.		Deaths.		Mortality per cent.
95	...	22	...	23·1

In various centres throughout the world, not only has much interest been created in the treatment, but the serum has been given a fair trial.

In the discussion at the Royal Medical Society of Vienna,

Dr. Unterholzner gave the following figures of cases treated in the Leopoldstadt Children's Hospital of that city :—

TABLE X.

Ages.	Treated *with* Serum.		Treated *without* Serum.	
	Treated.	Died	Treated	Died.
Under 1 year, - -	2 ...	1 ...	2 ...	1
1 to 2 years, - - -	9 ...	5 ...	6 ...	6
2 to 3 ,, - - -	7 ...	2 ...	7 ...	6
3 to 4 ,, - - -	3 ...	0 ...	5 ...	4
4 to 5 ,, - - -	2 ...	0 ...	2 ...	2
5 to 6 ,, - - -	1 ...	0 ...	2 ...	1
6 to 7 ,, - - -	2 ...	0 ...	2 ...	2
7 to 8 ,, - - -	2 ...	0 ...	6 ...	1
8 to 13 ,, - - -	3 ...	0 ...	4 ...	1
Totals, -	31 ...	8 ...	36 ...	24

Before the Medical Society of Trieste, Dr. Germonig gave the following results of the treatment :—Of 224 cases of diphtheria treated with Behring's serum, the mortality was 20·3 per cent. The usual mortality in the Civic Hospital of that city, from 1886 to 1894, was 60 per cent. of the total cases admitted; and to demonstrate the type of disease from which the 224 cases suffered, he pointed out that of 65 cases treated without serum, 33—that is to say, 50 per cent.—were fatal.

As showing the great international interest in this new cure of an international scourge, let me draw your attention briefly to what is happening in other countries, as well as in our own, in reference to the institution of measures for the preparation of the serum. Let us take France first.

In December last the Chamber of Deputies of that country unanimously voted 100,000 francs to the Pasteur Institute towards the expenses of the production of the serum. From this institute, since September, 1894, 50,000 doses of serum have been sent out. In Paris itself an institute has been founded, in addition to the work at the Pasteur Institute, for this special line of treatment—the municipality undertaking to defray its original cost; and the municipality, with the Department of the Seine, undertaking its annual upkeep, which is estimated to amount to £800 a year. In various provincial centres, as Lille, Havre, Bordeaux, Lyons, and others, similar arrangements have been matured, and it is estimated that 140 horses will suffice for the whole of France.

In Italy, Milan has founded a Sero-Therapeutic Institute; Padua and Venice have combined to give a sum of £480 to enable Professor Bonome, of the Pathological Institute of the former city, to prepare the serum for experimental purposes, whereupon, should the report prove favourable, an institute will be founded at Padua. The Municipality of Rome, too, voted £40 to purchase serum at an early point in the introduction of the treatment.

In Germany, as we have seen, not only has the serum been extensively used, but it is being prepared in considerable quantity.

In Holland, Amsterdam has made arrangements to produce its own serum. In Spain, Ferrán is preparing the serum at Barcelona. In Algiers, the Consul-Général has voted £80 to begin the preparation; other grants, as required, are to follow. In Russia, the serum is being prepared at the St. Petersburg Institute of Experimental Medicine, and the demand for it is so great that in two weeks 2,500 bottles have been sent out. In Cuba, at the town of Havana, the serum is being made at the Bacteriological Laboratory in connection with the *Med. Chir. Chronicle* of Havana. In Austria, Professor Paltauf has been entrusted with its preparation for that country. In Cape Colony, the Government bacteriologist is making the serum, and the Government are maturing a scheme whereby, at different centres, the bacteriological diagnosis of the disease may be made.

In the United States of America, the House of Representatives have appointed a National Commission to investigate the treatment. In New York, the Board of Health have instructed their Medical Officers to prepare a plan whereby the values of the different anti-toxins offered for sale may be determined, with a view, at the same time, to prevent worthless material being sold; and the municipal authorities of that city have placed £6,000 at the disposal of the Board of Health towards the application of the treatment. The Massachusetts State Board of Health and the Board of Health of Boston are now preparing their own serum.

In our own country, the Local Government Board authorised Dr. Klein to make researches into the subject and to prepare the serum, which he has done, and is doing, after the method I have already described. The British Institute of Preventive Medicine, as has already been said, was the first in this country to prepare the serum. At present it has 21 horses under treatment, 20 of which are ready for use, and the serum now prepared is of such strength that "·0001cc., mixed with a quantity of toxine, fatal to

a guinea-pig in 24 hours, will prevent the recurrence of any symptoms." Dr. Ruffer has succeeded in perfectly drying the serum without any loss of its potency, so that, when about to be used, it can be re-dissolved in a small quantity of distilled sterilised water. The Goldsmiths Company of London have given £1,000 to the Joint-Laboratory of the English Colleges of Physicians and Surgeons, which is under the direction of Dr. Sims Woodhead, for the purpose of preparing the serum. The Metropolitan Asylums Board have also determined that the serum should be used in the treatment of diphtheria in the various hospitals under their management. To-day, the supply of serum in this country is ample.

The Bacteriological Diagnosis of Diphtheria.

There is always a certain series of cases happening annually within the experience of the medical profession regarding the exact diagnosis of which—as to whether they are diphtheria or not—uncertainty prevails. This is due to the indefinite character of the physical symptoms. Hence, on occasion, a family may be thrown into unnecessary alarm, or lulled into a false security. We are now, however, in the fortunate position of being able to have our suspicious and indefinite cases of this disease diagnosed definitely and absolutely by means of bacteriological investigation. This, too, can be done very speedily by the skilled bacteriologist; indeed, within 24 hours of the receipt of a bit of cotton which has been used to brush the throat, he is able to say conclusively whether or not the diphtheria bacillus is present or not. It will be obvious that this attainment is of the greatest possible importance, since it declares at once whether or not we have this enemy to do battle with. Not only will it be of great utility in private practice, but it will also prevent a case with suspicious sore-throat being placed at once in a diphtheria ward of an hospital, while all the time it is a scarlatinal sore-throat. The danger to both new-comer and the occupants is reciprocal, and may result in serious complications or protracted illnesses.

By this form of investigation, too, the physician is enabled to say definitely that a case which is convalescent is, or is not, free of infection by the absence of the bacilli from their usual position. The period of infectivity of this disease, as in other infectious diseases, is by no means constant. While it may be said that, in the large bulk of cases, this period does not extend beyond 21 days,

examination by this method has revealed the fact that the bacilli of the disease are still to be found in the air-passages of the person, who, from all the other symptoms, looked quite free of the disease. Bacilli have been found in the air-passages of convalescents at the end of *four* and even of *five* weeks from the commencement of the attack, thus showing that they are still *infectious* persons.

Again, this mode of investigation would also prove of great value where, for instance, diphtheria attacks one child of a household, and where, for various reasons, it is proposed to take the other children, who are apparently quite well, to other places, but one or more of whom may take the disease later in their temporary homes. Experience abundantly has established this latter fact. Now, bacteriological examination would clear up which of them were infected and which were not.

The Nature of the Bacteriological Examination.

Where there are patches of any kind on the throat, or even where there are none, all that is required by the physician is to brush the throat of the patient with a pledget of sterilised cotton-wadding, place the wadding in a sterilised test-tube, forward it to an expert bacteriologist, who, within 24 hours, will, in practically every case, be able to say whether or not the diphtheria bacillus is present in the throat, because, when placed under suitable conditions, it grows so rapidly in appropriate culture media.

The value of an institution wherein such examinations can be made at a moderate charge is alike great to the public and to the medical profession, and provisions for such have already been made in the laboratories of the British Institute of Preventive Medicine and that of the Colleges of Physicians and Surgeons of London. Such an institution would be of the greatest importance and value in Scotland, whether undertaken by private subscription or by municipalities.

Hitherto Glasgow has been in the forefront of every sanitary movement in this country, and, probably, there is no tax which is paid more cheerfully than that for the purpose of maintaining and conserving the health of its inhabitants collectively; but in this department nothing has yet been done. However, many of us, who are interested in public health schemes, are hopeful that, in the new Sanitary Chambers, there will be found, as one of its

most important departments, a laboratory presided over and directed by a skilled bacteriologist, wherein such important investigations as have been indicated would be carried out. Such a laboratory would be invaluable, and would lead to much valuable life being saved.

If a precedent be desired for such a laboratory as this, instituted and maintained under a municipality, let me direct your attention to what the municipal authorities of New York have done and are now doing. So far back as 1887, the Port Health Authorities of New York instituted bacteriological examinations for the diagnosis of cholera, which were reported to be of "incalculable" service. In 1892 the City of New York followed their example by establishing a bacteriological laboratory. Out of this movement the investigation of doubtful cases of diphtheria followed as a matter of expectation, and, in 1892, examinations of about 4,000 cases of the disease in the health hospitals were made.

Dr. Herman Biggs, at the Congress of the British Institute of Public Health held last year, gave a full account of the work done in this particular direction in that laboratory, and his paper was published in the *Journal of State Medicine* for November, 1894 (Vol. II., November, p. 267). He pointed out in his paper that the value of such work was priceless, and every opportunity was afforded the medical profession of that city to have preliminary bacteriological examinations made by the officials of the laboratory of all doubtful or suspected cases of diphtheria. That this was recognised as a great boon by the medical profession, and as showing also the need that exists for such an institution, he pointed out that, in the first year of the operation of the scheme, 5,611 cases had been made the subject of examination, of which about two-thirds were proved to be true diphtheria. The New York Board of Health was, therefore, the first in the world to take this important step in advance. This example has since been followed by other large cities, by the Marine Hospital Service, and by the Medical Corps of the Army of the United States, and it is worthy of being copied by large centres of population like our own.

Preventive Use of Serum.

The serum, hitherto, has been mainly used as a curative agent; but it may also be used as a *preventive* agent--that is to say, in the event of one young member of a family being seized with

undoubted diphtheria, it would not only be necessary to inject the serum into its body, but it would be highly expedient to inject smaller doses into the other children, who, perhaps not yet affected, may either have been exposed to the same source of infection, or may be in process of incubation from their sister or brother.

To a limited extent, this would be like vaccinating against smallpox where one person was seized with the disease, while those who were in contact with him did not as yet show signs of the disease.

There is this, at least, to be said in favour of its use as a preventive, that the operation is trifling, is harmless, and, if aseptic precautions have been taken, without risk.

Objections.

All the objections that have been urged against the new treatment may be divided into two main classes, namely :—

I. Those that have been made by persons who object to animals being used for any scientific purpose ; and

II. Those, by certain of the medical profession, to the effect that the use of the serum is not unattended with uncomfortable consequences, if not, indeed, grave complications ; and, further, that while it is admitted that the anti-toxin is antagonistic to the Lœffler bacillus, the disease which the bacillus produces is, they say, not diphtheria.

To the first class of objectors, it may be conceded that the aimless cruelty to animals, whether under the name of a scientific experiment or under any other name, is a thing which is, and ought to be deprecated, by all right-thinking persons ; but, on the other hand, where, in the saving of human life, the sacrifice of animal life is necessary, much stronger reasons must be produced to convince a right-minded person that such sacrifice is a sacrilege or a sin ; for, whether from a religious or an economic standpoint, human life is always the most important.

One cannot help thinking that the deputation which was recently headed by Lord Coleridge, and which protested against the use of animals for the production of the serum by the British Institute of Preventive Medicine, failed to serve a useful purpose when it attempted to interpose between the reasonable use of animals and the cure of one of the most fatal and most dreaded

scourges of the nineteenth century; not only so, but to be logical, it would require to protest against the use of the bodies of animals for the production of a protective vaccine against the diseases of the same class of animals, as is exemplified in anthrax.

As it is written in the Holy Scriptures, "Fear not, therefore, ye are of more value than many sparrows" (Luke xii. 7).

Of the second class of objections it may be said that, while it is true that the use of the remedy does cause, upon occasion, certain disagreeable consequences, such as nettle-rash, pains in joints, &c., it must, at the same time, be said that there has not yet been recorded a single case to which the death could be attributed to the serum when properly used; and we can safely neglect, in view of the very beneficial results which follow the remedy, those minor and transitory ills.

Experience of the serum has now abundantly proved that it exercises a directly curative effect on the disease, that from it we may reasonably expect lessened mortality, and the sparing of many useful young lives; and now, as Virchow put it, no physician would be justified in withholding its use from a patient suffering with the disease.