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NOTICES.

ALTERED SUBSCRIPTION FOR MEMBERS OF THE INCORPORATED SOCIETY M.O.H.

The attention of Fellows and Members of the Incorporated Society is called to the fact that dating from October, 1894, the subscription to the Society will be one guinea instead of half-a-guinea as hitherto. It must be noted that this will include the subscription as members of a branch of the Society, the guinea subscription being inclusive for all purposes.

It will be seen from this that there will be no very material increase in the subscription for those who are members of the parent Society and of one of its branches. There will, however, be considerable saving of trouble in collecting subscriptions, etc., as one subscription only will be payable. The subscriptions (and those for Associates, which remain half-a-guinea) should be sent to the Treasurer, Dr. KEMPSTER, Chesterfield, North Side, Clapham Common, London, S.W.

MR. J. PARRY LAWS has been appointed a Science Inspector of Schools in connection with the Education Department. We regret that Mr. Laws has abandoned his very close connection with science as applied to public health, and regret that the comparatively non-remunerative character of the excellent work done by Mr. Laws should render such a step necessary.

THE DIAGNOSTIC VALUE OF THE DIPHTHERIA BACILLUS.

BY

E. KLEIN, M.D., F.R.S. (Read before the Section of Public Health of the British Medical Association, 1895.)

THE occasions are not by any means rare when either from a clinical or pathological point of view a case of acute inflammation of the fauces, larynx, or nasal membrane cannot be trustworthily diagnosed as diphtheria, and likewise it is not a rare occurrence that in such doubtful cases the means of isolation and prevention of further spread, owing to that uncertainty of diagnosis, are imperfectly carried out. It is sometimes assumed that where there is no false membrane, there is no diphtheria. Experience proves, however, that cases of so-called simple sore throat or simple rhinitis have been followed by, or have directly given rise to, true membranous diphtheria. As medical officers of health you have been occasionally placed in a position of doubt whether cases of sore throat in children associating with other children in or out of school are or are not cases of diphtheria, and you must have experienced no less doubt about the period at which a case convalescent from diphtheria can again be freed from isolation. By saying this, I am only giving expression to truisms, with which you are well familiar. I am saying it, however, with the object of laying emphasis on the proposition which I am about to make, viz., that in the majority of doubtful cases it is possible to decide whether the case is one of diphtheria, if in the secretion or membranous exudation of the affected mucous membrane the bacillus diphtheriæ, or the Klebs-Löffler bacillus, is demonstrable, and that this bacteriological diagnosis is in most instances verified by subsequent events.

Those who have had experience in examining the false membrane of true diphtheria cases, that is, of cases which from a clinical and epidemiological point, are at once recognised as such, agree that in some of these cases the superficial layers of the necrosed mucous membrane, that is of the false membrane, contain large numbers, occasionally in almost pure culture, of one and the same species of bacilli, aggregated in larger and smaller masses, separate, but more often grouped together. These are the Klebs-Löffler bacilli. From the superficial parts of the membrane the groups extend, as vertical sections show, also into the deeper parts of the membrane, and even as small groups into the inflamed but as yet not necrosed part of the mucous membrane. Cultivations made from such false

membrane, particularly from washings of it, yield an almost pure crop of the characteristic colonies of the diphtheria bacilli. A film or a cover-glass specimen made from a piece of the membrane shows a large majority of cylindrical or rod-shaped non-motile bacilli of about the size of 1.5 to 3.5 micro-millimetres, many distinctly conical, and pointed at one end; they occur as single rods or in pairs, attached end to end, in this latter case often touching with the thicker parts; occasionally a bacillus is seen which at one end is enlarged and club-shaped; sometimes a longer rod is met with which is composed (linearly) of unequal joints, some short, like granules, others longer and rod-shaped. Besides isolated bacilli there are often found irregularly grouped masses. As mentioned above, cultivations made by rubbing a trace of such a membrane, or by spreading a drop of sterile salt solution or bouillon in which a piece of the membrane has been shaken up, over the surface of solidified Agar or solidified blood serum or nutrient gelatine, yield colonies of one and the same kind of bacilli, viz., the Klebs-Löffler bacillus. On solidified blood serum (pure, or better, Löffler's serum) or solidified beef, bouillon Agar, on glycerine Agar incubated at 37° C., there are seen already, after twenty-four hours, whitish grey dots, which under a lens appear finely granular, and in transmitted light thicker and brownish at one point (generally in or near the centre) more transparent around this point; after forty-eight hours the colonies have increased in size, and the difference between a central thicker, less transparent, and a peripheral, more transparent part is more pronounced; after three or four days the colony being much larger shows in reflected light the central part prominent and yellowish, the peripheral part thin and comparatively film like. Microscopic specimens made of young colonies show that they are composed of the same cylindrical bacilli described above, many pointed at one end, some distinctly swollen and clubbed at the other; after forty-eight hours' growth there will be recognised longer forms with segregated protoplasm, being seemingly composed of a chain of rod-shaped bacilli with here and there a granule between them; these longer forms possess an enlargement either at one or both ends. Cultivations on gelatine incubated at 20.5, 21.5, or 22° C., show the same characteristic colonies, only the growth is much slower, but becomes less slow in further subcultures.

Such are the bacilli found in the false membrane of typical cases of diphtheria; as a rule these bacilli occur abundantly in the membrane, in some cases in almost pure culture. As regards the size (or length) of the bacilli both in preparations of the membrane as in those of the resulting colonies in culture there appear certain differences; for in some cases the bacilli are very short, in others there are a good many long forms isolated or arranged more particularly in chains—with segre-

gated protoplasm—and with larger or smaller club-like enlargements. From my experience I am not able to confirm the observations of those who recognise a definite relation existing between the size (length) of the bacilli and the severity of the case from which they are derived. I have seen cases which ran a rapidly fatal course, and on examination of the membrane taken from the fauces in faucial diphtheria, or from the larynx in diphtheritic croup, it (the membrane) contained in almost pure culture sometimes very short, sometimes long forms of the bacilli, and the same applies to the cultures made from the membrane; on the other hand I have examined membranes removed from the fauces of cases of diphtheria which were mild and recovered, and which yielded abundance of diphtheria bacilli of the short variety, while others yielded those of the long variety.

A second group comprises cases, which in clinical and epidemiological respects are undoubted diphtheria, which can be traced to an antecedent case of diphtheria, and in which the membrane or mucopurulent secretions contain a very variable number of the diphtheria bacilli; but they contain at the same time a very large number of other microbes, which in cultivation prove to be chiefly cocci—staphylococcus aureus and albus, small streptococci and chains of larger cocci. In these cases the number of diphtheria bacilli may be sufficiently large to be identified by their shape and grouping, and to be easily recovered by culture, or they may be so limited as to be only identified by culture; but even then the chances of identification are not great, if the bacilli are practically swamped by cocci, notably streptococci; in these cases only after careful and extensive examination can the colonies of diphtheria bacilli be identified, and I am under the impression that it is such cases which have given rise to the opinion, occasionally expressed, that in some diphtheria cases no diphtheria bacilli have been found.

The third group of cases are those in which no obvious antecedent connection can be established, and in which the clinical history does not enable one to make a diagnosis. It is obvious that it is precisely in these cases that correct diagnosis is of the utmost importance. Now, if from the secretion of the fauces (in cases of faucial inflammation, follicular tonsillitis), or from the nose (in cases of rhinitis), diphtheria bacilli can be isolated, then the case must be pronounced to be one of diphtheria. In some such instances, and in conformity with other observers, I have found the diphtheria bacilli at an early stage, and the diagnosis was confirmed by subsequent events; viz., other cases of true membranous diphtheria followed in the same household, and the first individual developed afterwards symptoms of paralysis. I may mention here the case of a child which sickened with "feverish

sore throat;" the child had been at a school where diphtheria had been prevalent, but it had been removed from it and kept at home for at least three weeks previous to the onset of the attack of sore throat. There was a little purulent discharge from the right tonsil, but no membrane. The child was seemingly again convalescent after three or four days. The examination of the discharge from the tonsils during the acute stage yielded under microscopic examination, as well as on cultivation, diphtheria bacilli amongst a large number of staphylococcus aureus. The diagnosis was pronounced as diphtheria; the attending medical man had at first some doubts about the correctness of the diagnosis. Some weeks later, the child complained of difficulty in swallowing, and on examination, paralysis of the fauces was established.

In another instance, from a case of sore throat with no previous history, a particle of membranous exudation was submitted for examination, and also here diphtheria bacilli were found; the case was mild, but was the starting-point for two subsequent cases of typical membranous diphtheria.

Owing to the scarcity of bacilli in some such cases the diagnosis becomes sometimes extremely difficult.

These cases are obviously of importance. In such cases occasionally only by cultivation, and then only by very extensive searching, can colonies of the diphtheria bacilli be discovered. In those cases in which, owing to the presence of a great number of other bacteria, chiefly cocci, the ordinary methods of tube cultivation are likely to fail in yielding evidence of the presence of the diphtheria bacilli, the following method of culture seems better adapted for demonstrating the diphtheria bacilli:—If, on the examination of stained cover-glass specimens of the membrane or other secretion, no definite evidence can be obtained of bacilli morphologically resembling the diphtheria bacilli, and if there are present large numbers of cocci and other microbes, Agar plates are made in this manner: sterile bouillon Agar in a tube is melted over the flame, and then poured out into an ordinary sterile plate dish. After the Agar has set, a piece of the membrane or secretion is rubbed, by aid of a platinum loop, gently over the solidified Agar surface in the whole extent of the plate dish, and the plate is incubated at 37° C., lid downwards. Next day the surface is found covered and bestrewn, as it were, with minute colonies; in many parts they are so numerous as to lie very close together. Now all these colonies are on the surface, none in the depth, because the sowing had been performed on the surface of the Agar already set. In an ordinary Agar plate, *i.e.*, when the melted Agar is inoculated, shaken, and poured out into a plate and allowed to set, a good many colonies of course come up in the depth, and only a fraction on the surface; and it is these latter

only which can be subjected to further microscopic and cultural examination; those in the depth are practically lost for such examination. Cultivations on the surface of Agar or serum contained in tubes offer likewise great difficulties if very numerous colonies are present, because if amongst a crowd of colonies only a limited number of diphtheria colonies are present, these obviously are easily overlooked.

Now, these difficulties are overcome by the above surface Agar plates, for in these almost every colony can be subjected to microscopic examination. Provided the sowing has been done by lightly rubbing the material over the surface of the Agar in the plate, all colonies are easily taken off by pressing against the surface of the Agar a cover-glass, *i.e.*, making impression preparations. I therefore proceed in this way that I make impression preparations on a series of cover-glasses from the Agar surface—if necessary, from the whole surface. The cover-glass impressions are dried and stained; each is found covered with great numbers of dots, each dot being a colony. Subjecting the dots on the cover-glasses to an examination with a power of 150—300, those that are made up of cocci can be easily distinguished from those made up of bacilli, and each one made up of bacilli can, with high powers, be readily identified as a colony of diphtheria bacilli by the shape and arrangement. Such plates, after having been used for impression specimens as above, are of no further use; no subculture can be made from them, but I think anyone with a little experience could recognise, when examining the Agar surface with a magnifying power of 100—150, whether a colony is composed of diphtheria bacilli, and if so, could, under a single microscope, make a subculture from such a colony.

Those cases where this method is necessary are not common; in most instances, though cocci are numerous, there are generally a sufficient number of diphtheria bacilli present to yield fairly easily recognisable colonies on the surface of Agar or serum in tube cultivations. I have had, however, recently two cases of what clinically and epidemiologically were clearly cases of diphtheria, but in which the tube cultivations failed; the above surface Agar plates yielded a positive result, in the cover-glass impressions amongst about each sixty colonies of cocci, one distinct diphtheria colony was identified.

Lastly, there are cases of sore throats without or with membranous exudations, in which even on most careful search no diphtheria bacilli can be recognized by culture. The purulent and membranous exudation in these cases is in morphological respects somewhat different from the true diphtheritic false membrane, inasmuch as it is composed of leucocytes only; it does not contain the reticulated fibrinous matrix constituting the true diphtheritic false membrane; besides, in the former

when examined in the fresh state by means of films, the number of microbes, though sometimes comparatively limited in number, consist chiefly of cocci, and cultivations do not yield diphtheria bacilli. The cocci chiefly present in these cases are *staphylococcus aureus* and *albus*, and particularly streptococci. Such cases are recognised now as cases of pseudo-diphtheria, or cocco-diphtheria; though in clinical respects they are not distinguishable from mild cases of diphtheria. As regards their sequelæ, they are not difficult to distinguish from true membranous diphtheria, and as stated before, they do not contain the true diphtheria bacilli.

The culture test is also of importance in respect of the disappearance of the diphtheria bacilli from the previously diphtheritically affected mucous membrane, or with regard to the presence or absence of these bacilli in the throats of persons that have been attending on, or have come in contact with, diphtheria cases; for if on diligent search by cultivation the bacilli cannot be demonstrated, it may be assumed that they are not present, though one should not be satisfied with a single examination.

On the whole, then, the microscopic examination and culture test alone will be found to supply a valuable help in diagnosis, and where necessary it may be effectually controlled by animal experiment. If a guinea-pig be inoculated subcutaneously in the groin with a broth culture, forty-eight hours incubated at 37° C., derived directly from a first subculture of a typical diphtheritic membrane, it will be found that as a rule 0.25 to 0.5 cc. is sufficient to kill a guinea-pig of 400 to 500 grms. weight in thirty to thirty-six hours. Already the morning after inoculation there is a distinct, soft oedematous swelling about the seat of inoculation, in some cases extending over large areas—the whole abdomen and chest; the animal is quiet and does not feed; it becomes gradually weaker in its movements; the temperature falls below normal, and the animal is found dead in thirty, or thirty-six, or at the latest in forty-eight hours. On post-mortem examination there is a hæmorrhagic, oedematous infiltration of the subcutaneous and muscular tissues in the region of the tumour, the nearest lymph glands are swollen and congested, the viscera, particularly the suprarenals, and also the kidney are congested. Cultivations from the tumour and the nearest lymph glands yield a copious growth of the diphtheria bacilli, the blood and viscera as a rule yield no growth. If the animal survives two or three days, it will be found that the tumour becomes more circumscribed and firm, and in a few days, or it may be a few weeks, it may begin to slough. The tumour of the later stages shows the same kind of necrosis as the diphtheritically affected mucous membrane in man.

According to the age and origin of the culture the result is found different as regards the severity and duration of the disease in the guinea-pig.

According to my experience, which is in harmony with that of other observers, there is no definite relation between the virulence of the culture, judging by the quantity required for producing a result in the guinea-pig, and the severity of the case from which the culture is derived.

Abbot states that he has found all transitional states between non-pathogenic diphtheria bacilli and those that had great virulence on the guinea-pig; I have not met with diphtheria bacilli—that is, bacilli which were derived from a presumable or a definite case of diphtheria and which morphologically and in culture (serum, Agar, gelatine, and broth) had all the characters of diphtheria bacilli—which in early and recent subcultures did not possess virulence on guinea-pigs, though I have met with bacilli which morphologically resembled the diphtheria bacilli, but in culture differed from them in some (it may be but slight) respects, and which did not act harmfully on the guinea-pig. I must, therefore, for the present withhold my judgment as to whether these are transitional forms or varieties of the true diphtheria bacilli.

Whether the absence of pathogenic action from some bacilli which are in morphological and cultural respects similar to the diphtheria bacilli, is sufficient to consider these bacilli as non-diphtheritic, I am unable to say from my own experience; I do not think that the subject is sufficiently exhausted; those bacilli which I have met with, and which in morphological and cultural respects were typical diphtheria bacilli, had pathogenic action; those non-pathogenic bacilli which I have met with, and which resembled morphologically diphtheria bacilli, showed some differences in cultural respects.

The true diphtheria bacilli are capable of producing in animals necrotic changes and pseudo-membrane with multiplication of the diphtheria bacilli in them, as, for instance, when inoculated on abraded and inflamed mucous surfaces in the guinea-pig, *e.g.*, the mouth, trachea, and vagina.

Add to this the important discoveries by Dr. Sidney Martin as to the chemical identity of the toxins of diphtheria, both of culture and of the human body infected with diphtheria, and as to the identity in physiological action of these toxins, their capability of producing paralysis; and I think we are justified in concluding that morphologically, culturally, experimentally, and in chemical respects sufficient data exist, which enable us to say that the Klebs-Löffler bacillus is the true cause of diphtheria, and to give in most cases a definite, positive, or negative opinion, according to the presence or absence from the exudations of the diphtheria bacilli; and consequently, if in cases which, in clinical, pathological, and epidemiological respects, are of doubtful nature, the presence of the true diphtheria bacillus is demonstrated, we are justified in declaring that they are cases of true diphtheria.