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BACTERIOLOGIC EXAMINATION IN THE DIAGNOSIS OF DIPHTHERIA.¹

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A GREAT deal has been said on the laboratory-technique and the value of bacteriologic examination in the diagnosis of diphtheria, yet the subject is of sufficient interest and importance to warrant a further explanation of the methods used in culture-examinations. It would seem unnecessary to go into the history of the organism further than to state that it was discovered by Klebs in 1883, and first artificially cultivated by Loeffler in the year following. The germ is generally known as the Klebs-Loeffler diphtheria-bacillus, and it has been fully proved to be the etiologic factor in the production of the disease.

It is generally admitted by all clinicians of experience that it is often impossible to make an accurate diagnosis of diphtheria, either from the clinical history, the anatomic lesion, or from both. There are no constant differences that separate the simple non-contagious forms of inflammation from the

¹ Paper read before the semi-annual meeting of the Erie County Medical Society, January 8, 1895.



diphtheric type, and it is in but a very small proportion of cases that an early and reliable diagnosis can be reached from any data obtainable. This was conclusively demonstrated in the examination of the cases of suspected diphtheria under treatment at the Willard Parker Hospital in New York, where the diagnoses were made by department diagnosticians and confirmed by the best medical talent. Subsequent bacteriologic examination in these cases revealed that from 30 to 50 per cent. were not diphtheria, but maladies of non-infectious character. Appreciate sending a case of follicular tonsillitis to a hospital and having it placed in a ward with diphtheric patients! This person, with a reduced vitality, is exposed to one of the most dreaded diseases when suffering from a malady to which hardly one in twenty-five succumbs.

a. It is in just such cases that an early bacteriologic examination is of the greatest sanitary importance.

Furthermore, it has been proved that in cases in which the Klebs-Loeffler bacillus has been found virulent diphtheria has been transmitted to others, whereas the person carrying the contagion was hardly in a condition to be called ill.

b. It is the systematic bacteriologic examination of such cases that plays a most important part in the advancement of preventive medicine.

Again it has been fully demonstrated that the bacillus of diphtheria remains a varied length of time in the throats of persons convalescing from the disease, even after the disappearance of all membrane and constitutional symptoms. Such persons,

if allowed to go at large, are a constant source of infection, transmitting the disease to others, and in this way increasing the prevalence of the malady.

c. It is from the bacteriologic examination in such cases that the greatest practical benefits can be derived.

Early in January, 1893, through the energetic efforts of Dr. Herman M. Biggs, of New York, a communication was addressed to the Health Board of that city, recommending systematic bacteriologic examination for the diagnosis of diphtheria. After some unavoidable delay, bacteriologists were appointed in accordance with the recommendation, with such assistance as was necessary thoroughly to carry on the work. From the time of the inauguration of this scheme those in charge had very little doubt of its ultimate success, but there were a few who doubted its practical efficiency. Several depots were established in various parts of the city, where culture-sets could be procured, and every step was taken to increase the facilities for early examination. Circulars were printed and sent to physicians, asking their hearty co-operation in the movement, and explaining the great necessity for such examinations. During the first few weeks the cases examined were comparatively small, but they have continually increased in number until at the present time the practice is universal.

In order to make clear the methods used in such examination it will be necessary to describe briefly the materials required. The culture-medium most suited for this work is a preparation of blood-serum after the formula of Loeffler. Pure serum is obtained

by allowing the freshly drawn blood of a young sheep or ox, contained in a wide-mouthed sterile jar, to remain twenty-four hours in an ice-chest. By that time the fluid part of the blood has separated from the solid portion, and is easily drawn off by means of a pipet. This serum is mixed in proportions of three to one of a 50 per cent. neutral sterile beef-infusion, containing 1 per cent. peptone, 1 per cent. dextrose, and $\frac{1}{2}$ per cent of common salt. This mixture is then poured into small sterile tubes (putting about 2 c.c. in each tube) and carefully sterilized in a blood-serum sterilizer, at a temperature of about 160° F. The tube is put at an angle during the process of sterilization so that after the coagulation of the blood-serum mixture one will have the greatest possible surface for the planting of bacteria. After these tubes have been rendered absolutely sterile they are ready for use. A sterilized tube containing a swab goes with each culture-tube, and these tubes compose the so-called culture-set.

How to make the primary inoculation is explained by a printed slip accompanying each culture-set. The slip, as supplied by the Department of Health in this city, reads as follows:

DIRECTIONS FOR MAKING CULTURES IN CASES OF SUSPECTED DIPHTHERIA.

The patient should be placed in a good light, and, if a child, properly held. In cases where it is possible to get a good view of the throat, depress the tongue and rub the cotton swab gently, but freely, against any visible exudate.

In other cases, including those in which the exudate is confined to the larynx, avoiding the tongue, pass the swab far back, and rub it freely against the mucous mem-

brane of the pharynx and tonsils. Without laying the swab down, withdraw the cotton plug from the culture-tube, insert the swab, and rub that portion of it which has touched the exudate gently but thoroughly back and forth over all the surface of the blood-serum. Do not push the swab into the blood-serum, nor break the surface in any way. Then replace the swab in its own tube, plug both tubes with the cotton plugs, and return the culture-set at once to the Health Department.

A report will be given by telephone the following morning, or can be obtained after 12 noon.

As soon as the tubes arrive at the department, that containing the inoculated blood-serum is placed in the incubator at a temperature of 36° C., and allowed to remain until sufficient growth appears to permit of examination. This requires a period of from twelve to twenty-four hours. After the proper time has elapsed a cover-glass preparation is made from the growth in the tube, stained with alkaline methylene-blue solution, and mounted in the usual way with Canada balsam. On microscopic examination of such a preparation with a 1-12 oil-immersion lens—if it is from a case of true diphtheria—the following picture will present itself: A large number of rod-shaped organisms stained blue, varying somewhat in length, about the same size as the tubercle-bacillus, but twice as thick, frequently clubbed and spindle-shaped, and almost invariably irregularly stained, presenting heavily stained segments in a lightly stained background. This is the bacillus of diphtheria.

It is claimed by some that the recognition of the bacillus is extremely difficult, owing to its resemblance to the germ described by Hoffman. It is the general opinion, however, that with thorough

examination of the bacterial growth, as described, which has been carefully inoculated according to the rules given, and kept at the temperature of the body, the microscopic examination can be, without doubt, relied upon. Of course, the majority of tubes sent in for examination will not reveal any bacilli, containing simply the common cocci found in the throat. In such cases a positive diagnosis is extremely easy. It was thought at one time that the size of the germ had to do with its virulence, but this is now generally conceded not to be the fact.

Much has been said by some authors as to an organism that by its culture-peculiarities and microscopic appearance is identical with the Loeffler germ, yet does not possess pathogenic properties when injected into susceptible animals. The name given to the organism is the "pseudo-diphtheric bacillus," and, as expressed by Abbott and other prominent workers in this field, it seems certainly to be a misnomer, for it is most possible and probable that this organism is none other than the true diphtheric germ, attenuated in virulence, or, as circumstances have rendered it, a non-virulent diphtheric bacillus.

It would appear to be far better to term "pseudo-diphtheric" those organisms that are enough like the true bacillus to attract attention, yet are distinguishable from it by certain morphologic and cultural peculiarities aside from the question of virulence.

It is a well-known fact that many pathogenic organisms, conspicuous among them being the micrococcus of sputum-septicemia and the so-called hemorrhagic septicemic group, undergo marked

variations in the degree of their pathogenic properties, yet such organisms are not designated as "pseudo," but simply the organisms themselves, having become attenuated in virulence.

Loeffler, himself, found the Klebs-Loeffler bacillus present in the case of a perfectly healthy child. Was the germ found virulent? Was it the germ of true diphtheria, or was it the resistance on the part of the patient, the healthy secretions and conditions of the mucous membrane, which lessened its virulence in this particular case? It seems most reasonable that it was the latter. If not, why is it that frequently, on examination of cultures made from the throats of persons taking care of diphtheric patients, one finds the germ present, and yet these persons present no constitutional disturbance. It is because the proper conditions are not present for the development of the disease, whereas others receiving the infection from an attendant might contract the disease in its most deadly form.

There happens in a certain number of cases which clinically are diphtheria, that the first inoculation will fail to show any diphtheria-bacilli. These cases, however, are exceptional, and it is not usually the fault of the bacteriologist that such is the case, because there are certain factors that should be taken into consideration:

1. The inoculation may have been made at so late a period in the disease that it is possible that the diphtheria-bacilli, though now absent, were present at an earlier time.

2. The growth on the culture-medium may be so scanty that it is probable that the inoculation was

not properly made, or that some antiseptic had been applied to the throat shortly before obtaining the material for inoculating the tube, sufficient of the antiseptic remaining to prevent growth on the serum.

3. The culture-medium may be badly contaminated and a positive recognition of any one organism be impossible.

4. The serum in the tube may have become too dry before the inoculation to permit of the growth of the diphtheria-bacilli.

In all these cases a second inoculation should be requested by the bacteriologist before giving a definite opinion as to the diagnosis.

Of the primary cultures examined at the Health Department in this city since the 1st of March, 1894, sixty-five were found to contain the Klebs-Loeffler bacillus and ninety-nine were found not to contain that organism. This makes a showing that about 39 per cent. of the cases were true diphtheria.

One case examined during that time of peculiar interest was that in the practice of Dr. Eugene Smith, of this city. Dr. Smith on the 18th of December, 1894, inoculated a tube from the pharynx of a child suffering with a malady characterized by great toxic disturbance, and, as he thought, possibly diphtheria. The tube was sent immediately to the Health Department and placed in the incubator. After twenty-four hours there was no apparent growth on the blood-serum, and a second inoculation was requested. The result of the second inoculation was like that of the first, there being no apparent growth after the twenty-four hours in the incubator. On microscopic examination of the swab it was found

to contain a considerable amount of a glistening whitish exudate. Two side-mountings were made of this exudate, one being stained with methylene-blue, and the other by Gram's method. On microscopic examination of these slides there was found to be present an organism having a distinct encapsulation and resembling morphologically the organism described by Fraenkel as being the etiologic factor in the production of pneumonia. The result of the examination was communicated to Dr. Smith. That night the child developed characteristic pneumonic symptoms, and is now convalescing from the disease. The primarily inoculated tubes were left in the incubator, and after the fifth day showed a growth consisting of several small clusters of isolated fine points, growing closely side by side, like those of the diplococcus of pneumonia. Some of the exudate from the swab was inoculated into several mice, and the animals died invariably inside of forty-eight hours of an acute septicemia.

All these points tend to show that the organisms found correspond with the Fraenkel germ, and it is of interest in that it fully explained why development did not occur on the blood-serum during the first twenty-four hours.

As to the points, in conclusion, which may be definitely stated, in relation to the bacteriologic examination in the diagnosis of diphtheria, there are the following:

1. A positive diagnosis of diphtheria can be reached in the majority of all suspicious cases by careful examining of the primary culture, inoculated as described, inside of twenty-four hours.

2. In cases in which the bacillus is not found there is little chance of infection.

3. In those cases in which the diphtheria-bacilli are found the cases should not be considered free from infective powers until subsequent bacteriologic examinations fail to reveal the organism.

NOTE.—During the discussion of this paper by various members of the Society an elderly and trusted physician brought up the old-time question as to whether diphtheria is primarily a local or a constitutional disease. He stated that he “believed that primarily it was always a constitutional disease, with perhaps sometimes coincident local manifestations,” *i. e.*, it was always the result of etiologic factors from within the body, and not a primary local affection.

With others I strongly contested this idea, and a heated argument ensued. Bacteriologists and men of experience should discuss this question in the current literature.

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