A STUDY OF THE BOAS-OPPLER BACILLUS.[1]

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(PLATE XVI.)

IN 1895, Oppler and Boas (1895 [1]) called attention to the presence of a large Gram-positive bacillus in the gastric contents of patients suffering from carcinoma of the stomach, and in the same year, Schlesinger and Kaufmann (1895 [2]) found this organism in nineteen out of twenty cases of the disease examined.

To the investigations of these workers very little has since been added, and the fact that the organism has never been thoroughly studied is due partly to the difficulty of its isolation and primary cultivation, and partly also to the involution changes which the organism undergoes in fluid media, leading observers to suppose that it has been overgrown or lost in culture. Further, the ordinary laboratory media are not suitable for the growth of the bacillus—milk, or media obtained from milk, being necessary for this purpose.

The Boas-Oppler bacillus is found in the stomach contents of the great majority of patients suffering from gastric cancer, especially in the later stages of the disease, and in these cases the normal hydrochloric acid of the stomach is usually absent, lactic acid being frequently present in its stead.

The occurrence, then, of the bacillus, especially in large numbers, forms one of the links in the chain of evidence in favour of malignant as opposed to benign disease, and bacteriological examination of the gastric contents is of practical value only in the differential diagnosis of these two conditions. That the organism may occur in non-malignant conditions of the stomach is true, and we have occasionally found in chronic gastritis large bacilli morphologically identical with the Boas-Oppler bacillus, but in these instances the organisms were extremely scanty. Considering the number and variety of organisms which must daily enter the human stomach, the relative freedom of this portion of the alimentary tract from bacterial flora must be assumed to be due

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to the bactericidal action of some normal secretion of the viscus—presumably the hydrochloric acid. In cases of cancer of the stomach, however, where the hydrochloric acid is absent or reduced to a minimum, the lactic acid which is present exerts an equally powerful inhibitory influence upon the growth of most bacteria.

There is one group of bacteria, however, described under the collective term "acidophile," the members of which are capable of producing lactic acid and of multiplying in a medium containing this substance in amounts detrimental to the vitality of almost all other organisms. According to Heinemann and Hefferan (1909 [3]), to whom we are indebted for the only satisfactory account of this group, it appears reasonable to suppose from the descriptions that one organism has been described by many authors, under different names. Thus, the *B. bulgaricus*, the *B. acidophilus*, the *B. gastrophilus*, the *B. bifidus*, and possibly the Boas-Oppler bacillus may ultimately prove to be one and the same organism. These bacilli are sometimes termed the long-rod group of lactic acid bacteria in contradistinction to certain other round or oval organisms which are also capable of producing lactic acid when grown in carbohydrate media. The representative member of the group, the *B. bulgaricus* or bacillus of Massol, first described by Grigoroff (1905 [4]), is widely distributed in nature, but has only recently been called into prominence, owing to the fact that it has been found to be the chief factor concerned in the production of soured milk.

In the course of our investigations it became increasingly evident that the morphological resemblances, and the power of multiplying in strongly acid media, brought the Boas-Oppler bacillus within the same category as the acidophile bacteria. Comparison was made with a known strain of the *B. bulgaricus* which was subjected to cultivation under exactly the same conditions as the organism in question, with the result that the differences were found to be insignificant and certainly not of sufficient importance to separate species.

The specimens of the Boas-Oppler bacillus used in the investigations were obtained from the stomach contents of three definite cases of carcinoma ventriculi where the organisms were present in abundance; free hydrochloric acid was absent and lactic acid present in marked amount. At first, difficulty was experienced in obtaining cultures of the bacillus owing to the readiness with which vitality is lost, especially in competition with other bacteria. It was found that isolation was most easily effected by sowing in whey, with subsequent transplantation after three days' incubation to "nasgar" plates, from which the characteristic colonies could be transferred to litmus milk. A word of caution is here necessary. After growth in fluid media, the bacillus alters so greatly in morphology and staining reactions, that unless on the alert one is apt to be deceived into thinking that it has been overgrown by extraneous organisms.

With a view to restraining other bacteria, Cohendy (1906 [5]), for

the isolation of the *B. bulgaricus*, recommends a medium containing 0·5 per cent. acetic acid. For both this organism and the Boas-Oppler bacillus, however, we have found that for all practical purposes the whey method is most suitable, as the bacilli are so active in producing lactic acid that in twelve hours they form sufficient acid to inhibit almost all other bacteria. Moreover, although capable of living and multiplying in lactic media, the organisms much prefer a neutral medium, the lactic acid being apparently merely a by-product.

As found in the stomach in cases of gastric cancer, the Boas-Oppler bacillus is a large, non-motile, non-sporing bacillus, with square ends, closely resembling the bacillus of anthrax, staining solidly with the usual anilin dyes; it is Gram-positive. It is found singly and in filaments of four or more distinctly jointed members which frequently assume angles with each other, giving rise to a zigzag or geniculate appearance. Fresh specimens are highly refractile, and when treated with Gram's iodine alone are turned yellowish-brown in colour, a useful point in distinguishing this organism from the Leptothrix buccalis which assumes a distinct bluish tinge. In culture, the Boas-Oppler bacillus is found to resemble the *B. bulgaricus* so closely that the following description of the morphological and cultural characters applies equally well to both organisms, with this proviso, that the *B. bulgaricus* is, until educated by one or two subcultivations in milk, somewhat slower in coagulating this medium.

MORPHOLOGY AND STAINING REACTIONS.—There is probably no other group of organisms which exhibits in culture such a variety of involution forms as the long-rod group of lactic acid bacteria. Films from cultures show great variations in shape and staining reactions of the bacilli, according to the composition and reaction of the medium used and the age of the growths. Clubbed, curved, granular, and filamentous forms are common in fluid media; and, particularly when milk is used, true dichotomy may often be observed, the organisms frequently assuming a Y shape. In growth on a neutral medium, especially if this be solid, the forms are shorter and stain more densely than is the case in media containing considerable amounts of acid.

In young cultures in whey, strongly Gram-positive filaments are the rule, but in older growths, where the percentage of acid is higher, long tortuous threads, staining partly with the gentian violet and partly with the counterstain, are found. In these cases, the protoplasm appears to have retracted within the sheath of the organism, leaving long lengths of empty covering which are liable to be mistaken for Gram-negative bacilli. When treated by Neisser's method, a tendency to metachromatic and polar staining is seen, and an appearance like spore formation is frequently evident. According,

then, to the age of a culture, all varieties of forms are found, but even in very old acid cultures, a transplantation to a neutral medium is always quickly followed by a return to the normal shape. Plate XVI. Fig. 1, indicates the more common involution changes which the organisms undergo in fluid culture.

CULTURAL CHARACTERISTICS.—The optimum temperature for growth appears to be above 37° C., and the organism exhibits a distinct preference for anaerobiosis. Milk is coagulated in about three days with the formation of a soft homogeneous clot. It is the most suitable medium for stock cultures, as in it the vitality of the Boas-Oppler bacillus is maintained unimpaired for considerable periods. In this medium as much as 3 per cent. of lactic acid may be formed, and if tinged with litmus a deep vivid pink is produced within three days, but later the colour is completely discharged, leaving a dead white coagulum. Cultures have an acid acrid taste and a distinctly sour odour. The particular strain of the *B. bulgaricus* investigated by Heinemann coagulated milk in from forty-eight to seventy-eight hours at 37° C., but Herschell (1909 [6]) has shown that by reinforcing the culture in special media this time may be reduced to ten or even eight hours.

Whey is a good medium for culture, and twelve hours after inoculation a definite but slight turbidity is apparent, and a powdery deposit—more marked in twenty-four hours—forms at the bottom of the tube. This deposit is found on examination to consist of long, tangled threads of the bacillus, healthy at first, but rapidly degenerating as acid is formed.

Twenty-four hours' growth on a solid medium, *e.g.*, "nasgar," shows characteristic small densely matted colonies with woolly edges which give an irregular contour. They are extremely minute, are best seen by reflected light, and resemble discrete streptococcal colonies, but have a decided bluish colour. So marked is this last feature that it distinguishes these colonies from those of all other organisms commonly found in the gastro-intestinal tract, and after a little experience one can identify with certainty the colonies of the Boas-Oppler bacillus in a mixed culture made from the stomach contents. Films from "nasgar" show bacilli which have, to a great extent, preserved their original form, namely, filaments in intact sheaths; but short single stumpy forms also occur (Plate XVI. Fig. 2).

Similar but less luxuriant growths may be obtained on an agar fortified with whey, acid, or ascitic acid fluid.

The ordinary laboratory media allow of little development, but by sub-cultivating from strong milk cultures and subsequent anaerobic incubation we were successful in obtaining growths on all the usual media, including glucose gelatin, and potato. *Broth* yields a poor growth with slight uniform turbidity and a flocculent deposit. Films from this latter show organisms resembling chains of pneumococci.

Glucose gelatin is not liquefied, and in stab preparations minute white specks appear along the line of inoculation in seventy-two hours, the colonies being more numerous towards the deeper end of the stab. On *potato* a white, viscid, almost invisible smear forms after three days' incubation. *Ordinary agar, glycerin agar, glucose agar,* and *serum* yield weak growths, the colonies of which resemble those obtained by culture on nasgar.

The organism does not produce indol, there is no fluorescence in neutral red broth, blood agar is not hæmolysed, and the Voges-Proskauer reaction is negative.

No gas is formed in any of the carbohydrate media. Lactose, galactose, inulin, and lævulose show the production of acid in three days. Saccharose, glucose, dulcite, mannite, sorbite, salicin, raffinose, maltose, and adonite are not affected.[1]

VITALITY.—The organisms are killed by half an hour's exposure to a temperature of 60° C.

PATHENOGENICITY.—Subcutaneous injection of 2000 million living bacilli into the arm produced merely a slight local reaction lasting three days. There was no general rise of temperature. A control injection of 2000 million of the *B. bulgaricus* produced a similar result. Agglutination and complement-fixation tests were not performed.

SUMMARY AND CONCLUSIONS.

1. Specimens of the Boas-Oppler bacillus were obtained from three definite cases of carcinoma of the stomach: in these cases free hydrochloric acid was absent, and lactic acid present in the stomach contents.

2. Isolation and primary cultivation were effected on special media, and the morphological and cultural characters were compared under standard conditions with those of a known strain of the *B. bulgaricus.*

3. The evidence went to prove that the Boas-Oppler bacillus is identical with the *B. bulgaricus,* and not an organism *sui generis.*

4. True dichotomy was observed in cultures both of the Boas-Oppler and of the *B. bulgaricus,* indicating that another member of the long-rod group of lactic acid bacteria, namely, the *B. bifidus* of Tissier, is probably identical with the above organisms.

5. The inference was drawn that in cases of cancer of the stomach it is the absence of hydrochloric acid that allows of the growth of the bacillus, and that the lactic acid is formed as a result of the activity of this organism.

[1] In these tests the purest Merck's sugars, 1 per cent. in peptone solution, were used, and in the reactions the *B. bulgaricus* gave the same results as the Boas-Oppler bacillus under standard conditions.

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DESCRIPTION OF PLATE XVI.

Fig. 1.—*A.* Forms found in the stomach contents. *B.* From solid cultures. *C.* From potato. *D.* Involution forms from old solid cultures.

Fig. 2.—*E.* Involution forms from whey and milk. *F.* From sugar solutions. *G.* Y-shaped form. *H.* Long filamentous forms from young cultures. *I.* Found in growths on acid media in old cultures. *K.* ? Spore formation. *L.* Coccoid forms from broth.

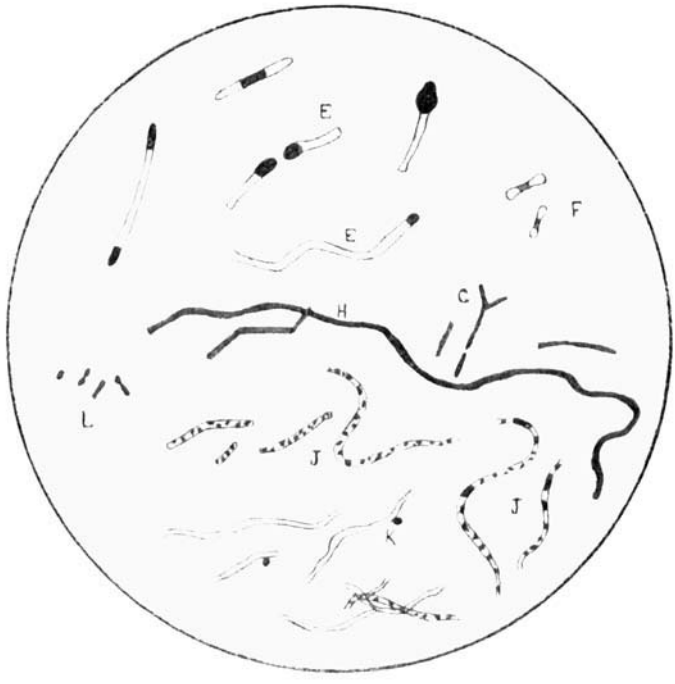

FIG. 1.

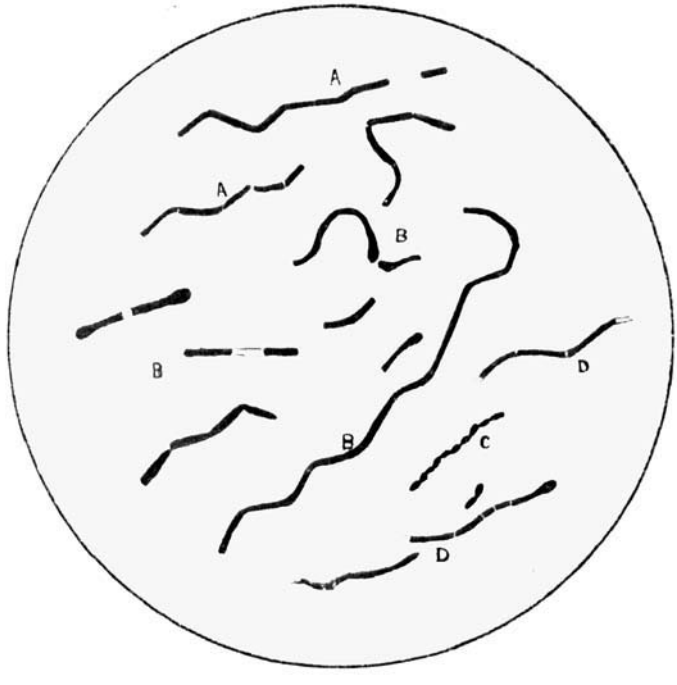

FIG. 2.