tical bone and periosteum) of the rib as used by Eloesser,[4] Whitman,[5] Kleinberg,[6] and Bisgard.[7]

7142 C

Certain Properties of Bacterial Mucus.

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It has previously been reported that certain strains of *B. subtilis* exhibit a peculiar phenomenon when grown on saccharose agar plates.[1] The colonies are stringy and contain besides the bacteria a large amount of apparently amorphous, mucoid material. This material under appropriate conditions spreads out from the colonies on the surface of the agar for a distance of 10 to 20 mm. forming a halo around the colonies. Bacteria cannot be found in the halo and transplants made from it do not grow on the usual agar plates or in broth. However, when the amorphous substance is transplanted to saccharose agar plates it grows in tiny transparent colonies. The detailed study of this phenomenon will be described in another place[2] and only a few properties of it will be mentioned here.

It has been possible to make only 3 consecutive transfers of the amorphous substance. The bacteria are not regenerated in these cultures. In the developing colonies of the bacterium the amorphous substance usually appears first as a capsule around a few of the bacteria which often appear swollen and disintegrating. Similar phenomena, although varying quantitatively in degree, may be observed in cultures of many different types of bacteria, but are usually seen only in the first few transplants after isolation.

It is known that under certain conditions various bacteria produce large amounts of mucoid substances. "Ropy beer" is a well known example of this. This phenomenon has been attributed to an excessive production of bacterial capsules. According to our obser-

4 Eloesser, L., *Arch. Surg.*, 1920, **1**, 428.

5 Whitman, A., *Am. J. Surg.*, 1929, **6**, 801.

6 Kleinberg, S., *J. Bone and Joint Surg.*, 1929, **11**, 66.

7 Bisgard, J. D., *Arch. Surg.*, 1933, **26**, 796.

1 Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1205.

2 Dienes, L., *Zbl. f. Bakt. I. O.*, in press.

vations the mucoid substance grows not only as a capsule around the bacterium but is also produced independently from the bacteria in the medium.

We tried to determine with several different methods whether the mucoid substance contains morphological elements which would account for these surprising growth phenomena. In many cultures, using dark field illumination or in dark background preparations as described in the previous note, the amorphous substance seems to consist of tiny granules. In other cultures, however, these granules were absent. The application of flagella staining methods to our cultures resulted in two observations of interest. (1) In certain bacilli 2 to 10 or more small round bodies are formed which later are liberated and sometimes are found in great numbers between the intact bacteria. These small bodies are certainly different from the previously mentioned ultramicroscopic granules and their significance is unknown. (2) Especially in young cultures, the seemingly amorphous mucoid substance consists of long fine interwoven filaments as shown in Fig. 1. Preparations from liquid cultures developing a stringy consistency show masses of these filaments. In a few cases we succeeded in demonstrating these structures in a young halo developing around subtilis colonies. With certain staining methods the bacteria in these cultures appear encapsulated but in preparations stained successfully by flagella staining methods the capsule resolves into a mass of fine filaments.

After these observations were made with subtilis strains we studied other bacteria including meningococci, gonococci, pneumococci, streptococci producing a mucoid growth, and influenza bacilli using flagella staining methods. The organisms were studied immediately after isolation from clinical material. In the case of all organisms mentioned above, the cultures contained masses of the filamentous structures. Fig. 3 shows the filaments from a culture of meningococci and Fig. 5 shows Type III pneumococci with capsules. In these cases we have not observed the growth of the filaments independently from the bacteria.

Similar filamentous structures were seen by several authors and Hinterberger described them exactly[3] in mucoid cultures of the anthrax bacillus and later in pyocyaneous cultures.[4] Zettnow observed them accidentally in the cultures of many bacteria.[5]

The most important question in connection with these filaments

[3] Hinterberger, A., *Zbl. f. Bakt. I. O.*, 1901, **30**, 417.

[4] Hinterberger, A., and Reitmann, C., *Zbl. f. Bakt. I. O.*, 1904, **37**, 160.

[5] Zettnow, E., *Zschs. f. Hyg. a. Inf. Kr.*, 1918, **86**, 25.

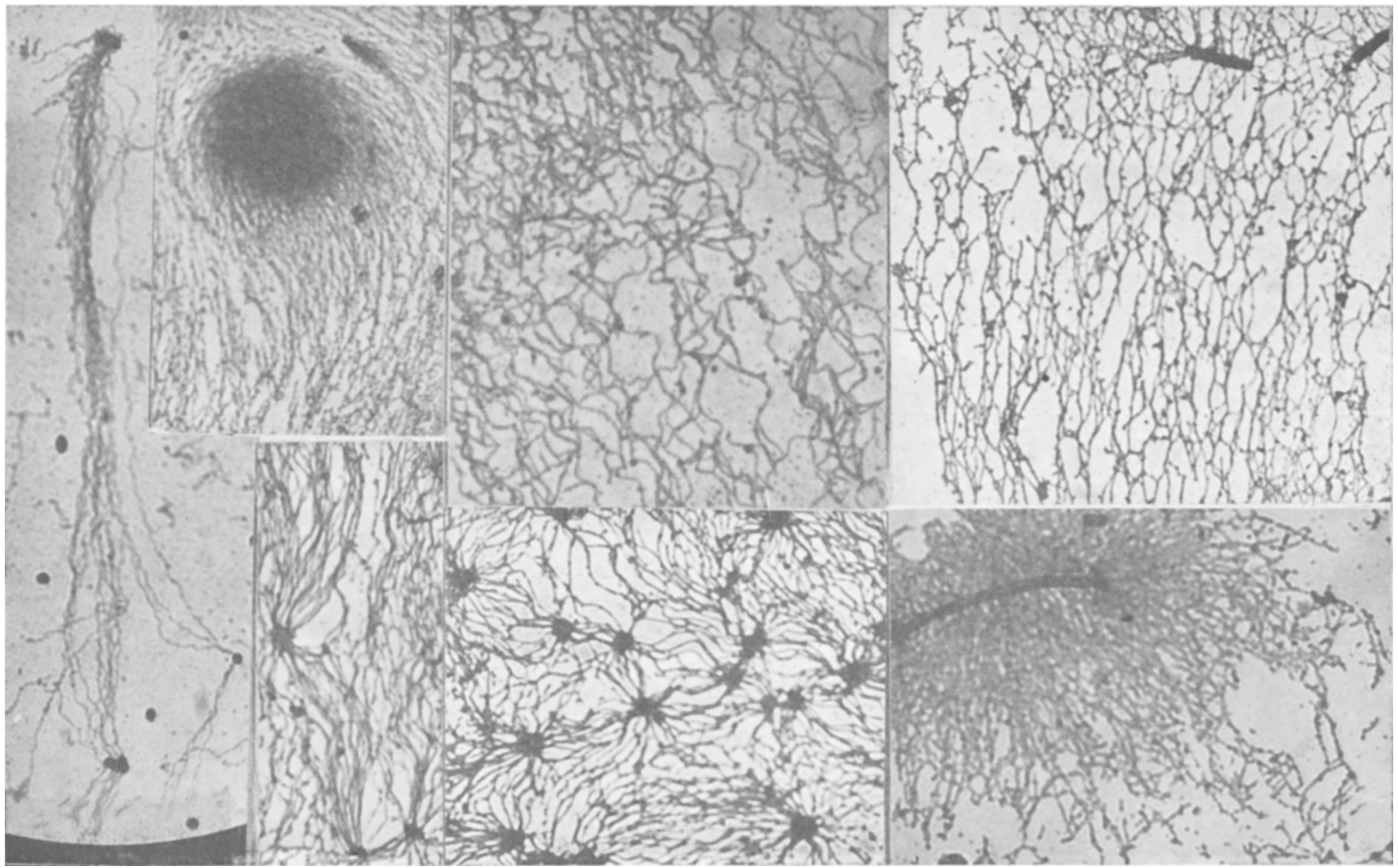

FIG. 5. FIG. 3. FIG. 1.

FIG. 7. FIG. 6. FIG. 4. FIG. 2.

All pictures are taken with the same magnification, 1 × 1000.

FIG. 1. Fresh culture of *B. subtilis* strain 9 on saccharose agar.

FIG. 2. The same bacillus from saccharose broth culture.

FIG. 3. Meningococci, saccharose agar. Second generation from spinal fluid.

FIG. 4. A Gram-negative bacillus, 3247, on saccharose agar.

FIG. 5. Pneumococcus type III rabbit serum saccharose agar.

FIG. 6. The Gram-negative bacillus 3247 from a ropy saccharose broth culture.

FIG. 7. The same bacillus from saccharose broth culture with long filaments.

is whether they represent structures actually present in the cultures or whether they are artefacts in the preparations. The evidence now at hand strongly suggests the former. They differ from artefacts which we see in other preparations of this type. Attempts to obtain similar structures from such substances as glue, gelatin, or a stringy solution of purified mucin were unsuccessful. On the other hand in preparations of spinal fluid from cases of meningitis and from sputum containing numerous bacteria it was possible to observe the same filamentous structures as found in the cultures. In preparations from spinal fluid the fibrin from a freshly formed coagulum appears in the form of long interwoven filaments which furnishes additional evidence that these structures also in the bacterium cultures are genuine. In preparations from bacterial cultures the filaments are often torn and ruptured as a result of smearing, indicating that the filaments were present in the liquid before it dried on the cover slip. It is also significant that the filamentous structures are characteristically different in preparations made from cultures of different bacteria whether grown on solid or in liquid media. Figs. 1 and 2 show the filaments from agar and broth cultures of *B. subtilis*. Figs. 4, 6. and 7 illustrate the same structures from similar cultures of a gram negative bacillus. The difference in the type of filaments produced by the 2 organisms is apparent. Artefacts usually vary in appearance and do not reproduce the same characteristic pictures as do these structures.

It would be early to express an opinion concerning the nature of the filaments and as to whether they are living structures or not. The formation of the halo and the development of tiny colonies in transplants from it strongly suggest growth phenomena. The possibility of the independent growth of the capsular substances which play such an important rôle in virulence and immunity gives a special interest to the study of the described filamentous structures.

Summary. In the cultures of numerous bacteria, and particularly in recently isolated strains, masses of fine filaments are present which are seen only in preparations stained with flagella stains. In stringy

broth cultures similar filamentous structures are present. The capsule is frequently seen to consist of a thick agglomeration of these filaments around the bacteria. In certain subtilis cultures the mucoid substance which consists largely of these filaments shows phenomena suggesting that it is capable of growth independently from the bacteria.

Notes on the staining methods: The filaments could be stained with all flagellar staining methods tried. The following technique gave most constantly satisfactory results. The agar or broth cultures are spread on a cover slip with the help of a small drop of water without being previously suspended or diluted and quickly dried. It is sometimes helpful to extract the preparation after drying with distilled water. For a mordant, Loeffler's solution is used (prepared a few weeks previously) and is applied for 2 to 3 minutes without heating. The preparation is then silvered with aethylamin silver solution. Not all samples of the mordant give satisfactory results.

7143 C

Kidney Function in Pregnancy. II. Effect of Posture on Diuresis.

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(Introduced by A. W. Rowe.)

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In line with observations on normal non-pregnant individuals as to the influence of posture on urine volume,[1] an opportunity has presented itself to carry out similar studies during pregnancy. One private patient volunteered to carry out a series of tests throughout her pregnancy. Commencing about 20 weeks before term, she did 4 tests per month until the time of delivery, and 2 series postpartum.

The tests were done in 4 positions, the horizontal back position, sitting up, the Trendelenberg position, and horizontal on the side. In the latter position the patient was allowed to turn on either side at will. Each monthly series included these 4 tests, and, so far as possible, the tests were arranged for every other day.

Physically Mrs. J. H. was a primapara in excellent health until the very end of her pregnancy. She was not subjected to any ex-

[1] Janney, J. C., Riley, G., and Walker, E. W., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 398.