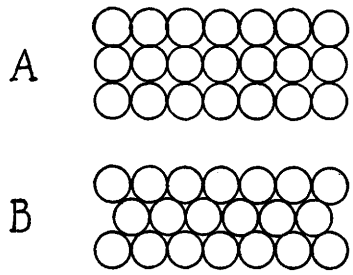

Fig. 1

Needless to say this is a highly simplified and idealized method of calculating the extracellular volume in muscle. No account, for example, has been taken of the effect of variation in the diameter of the fibers, nor of the effect of possible crowding and so distortion of the circular cross-section that has been assumed for the fiber. These and other factors, however, cannot be checked by reference to slides of actual cross-sections of muscle since the fibers suffer unpredictable alterations in the preparation of the sections. At any rate, the result obtained above is in striking agreement with Fenn's value of 14.5% for the chloride space, and in so far as the above considerations have validity they may be accepted as helping to confirm Fenn's conclusion as to the identity of the chloride space and the extracellular space in muscle.

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A Peculiar Reproductive Process in Colon Bacillus Colonies.*†

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In a previous publication attention was called to the fact that colonies resembling the L type colonies of *Streptobacillus moniliformis* develop in various bacterial cultures.[1] Observations to date

* The expenses of this investigation were defrayed in part by a grant from the Commonwealth Fund.

† This is publication No. 39 of the Lovett Memorial.

1 Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 636.

would seem to indicate that these L type of colonies originate from, or in close association with large swollen bacterial forms. If such observations have been interpreted correctly, then these large bacterial forms possess a definite biological significance. The occurrence of such large forms in bacterial cultures has been observed in the past. They have been regarded by the majority of bacteriologists as degenerative forms because they fail to multiply when transplanted.

In studying the L type of colonies, cultures of various bacterial strains were examined in the manner previously described *in situ* on the surface of the agar. If one examines such cultures from day to day one has an excellent opportunity of determining the fate of the large bodies. Our experience was similar to that of previous workers;[2] namely, that the large bodies disintegrate when transplanted without further development. During the course of this study, however, a single observation was made which seems sufficiently significant to warrant its being recorded.

Examination of a blood agar plate which had been inoculated with urinary sediment revealed large, intermediate, and tiny sized colonies. The larger colonies consisted of colon bacilli of the usual size and shape; whereas the tiny colonies were composed of long, wavy filaments showing all grades of transition into large fusiform or round bodies (Fig. 1). The large bodies were either uniformly stained or contained granules and vacuoles of different types. From Fig. 1 it is apparent that the large bacterial forms differ considerably from the normal appearance of colon bacilli. Such forms are present in small numbers in many freshly isolated colon bacillus cultures. In only 0.5% of the strains isolated from urine specimens did these forms appear in large numbers. These large forms are the result of the swelling of individual bacteria and bacterial filaments. They certainly do not represent the growth of an extraneous organism in the cultures. It was observed directly under the microscope that the filaments which produce the large bodies transferred on plain agar produce bacteria of normal shape. The study of transplants indicated also that the tiny colonies are genetically identical with the large colonies. The tiny colonies covered sufficiently large areas so as to render transplantation possible without contamination from the larger colonies. The first transplants on blood and ascitic agar plates again gave a mixture of tiny and large colonies. Subsequent trans-

2 Klieneberger, E., *Ergebnisse der Hygiene, Bacteriologie, etc.*, 1930, **11**, 499, Springer, Berlin.

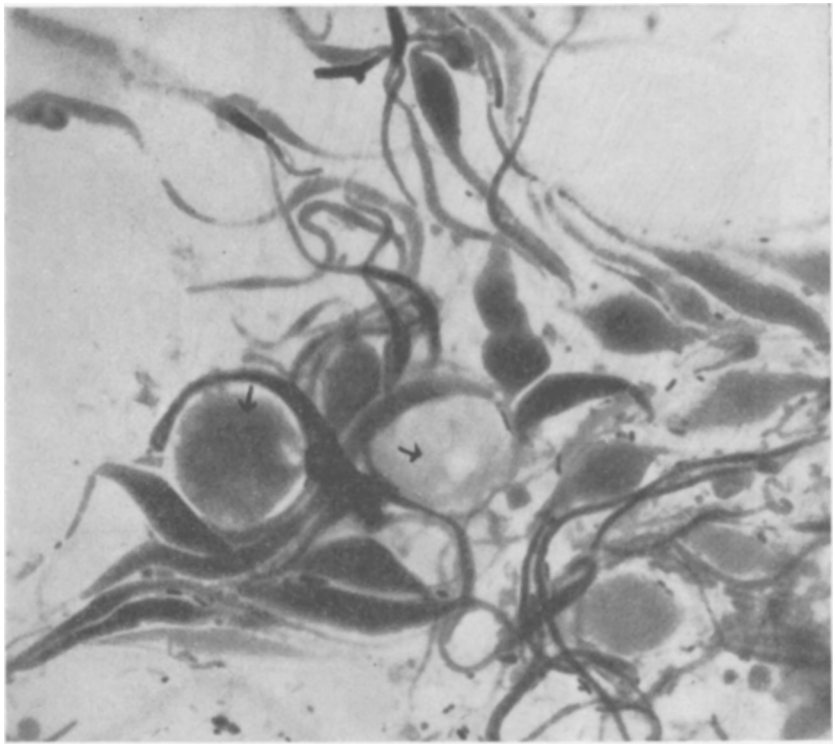

FIG. 1.

Fig. 1 represents a tiny colony showing transitory forms from filaments to large fusiform and round bodies. Two large bodies containing vacuoles are marked with arrows. Impression preparation from colonies stained with methylene blue on the surface of agar. (1:1000.)

plantation resulted in the appearance of normal appearing colonies. Transplants on plain agar produced normal sized colonies.

The original blood agar plate which had been inoculated with urinary sediment was retained and observed daily. During this period it was kept in the refrigerator except for several hours each day, when it was exposed to room temperature. Each day a small block of the agar was cut out, placed on a glass slide and stained with methylene blue. In the block removed on the sixth day 2 tiny colonies were seen which were different from the others. The large bodies in these 2 colonies appeared to be completely filled with small regular shaped bacteria. The membranes of these large bodies were intact and they differed from the large bodies in the other colonies only in the fact that their content was made up of bacteria. The whole colony consisted of these large round and fusiform bodies; bacterial growth outside of them was not present. The shape and appearance of these large bodies is very characteristic and cannot be

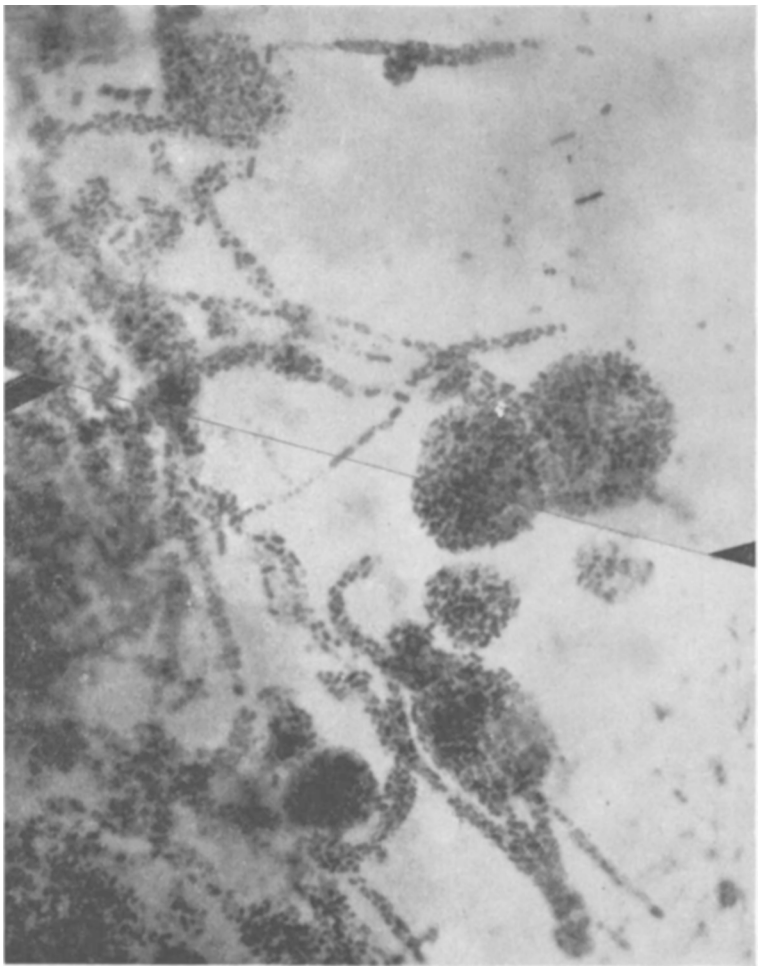

FIG. 2.

Fig. 2 represents a part of the colony in which the large bodies were filled with bacteria. Though the preparation was damaged it is apparent that the dense groups in which the bacteria are arranged imitate closely the shape of the large fusiform and round bodies. The whole colony consisted of similar structures. The fusiform body in the top center of the photograph shows in part the membrane which in the wet preparation surrounded all large bodies. (1:1000.)

mistaken for anything else. The preparation in this condition was seen by Dr. E. R. Sullivan and Dr. H. B. Arnold as well as by the author.

The making of a permanent preparation was only partially successful because the small agar block (approximately 3 x 6 mm) was crushed when the upper portion of the media containing the colonies was being cut off. In order to save the preparation the entire agar block was pressed into a thin layer on the slide and allowed to dry.

The membranes had disappeared in the dried preparation; however, the arrangement of the bacteria indicated clearly the shape of the large bodies. Fig. 2 is a photomicrograph of the dried preparation. Attention is called to the fact that the bacteria are arranged in the whole colony imitating the shape of the large bodies. Only in those places where the colony was injured during the preparation are the bacteria in formless masses.

It is interesting that all large bodies of the 2 adjacent colonies were filled with bacteria. The filaments from which the large bodies were produced were also fragmented into bacteria. Such transformations were never observed in thousands of these large bodies found in subsequent preparations made from the original plate and transplants. It is obvious that such transformation occurs rarely and then under conditions as yet unknown, but if these are fulfilled the transformation involves all the large bodies in a certain area of the culture.

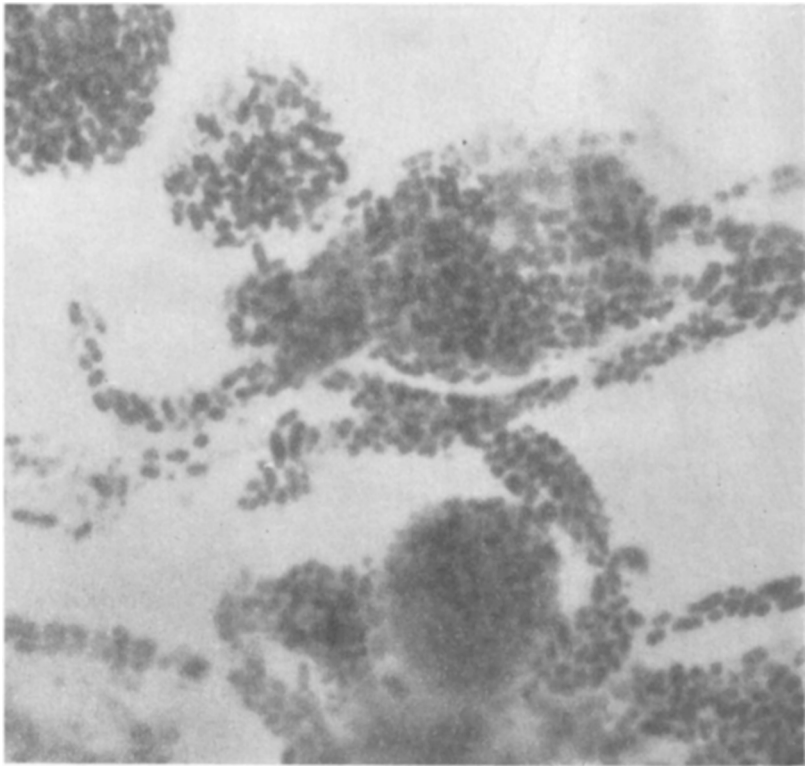

FIG. 3.

Fig. 3 gives details of Photograph 2 enlarged. In Fig. 3 the bacteria are plainly visible in the large bodies. (1:2000.)

The large bodies are very fragile structures and are readily destroyed by the slightest mechanical injury. It appears unlikely, therefore, that bacteria migrated into the large bodies from the outside and multiplied there without changing their shape. It would seem also unlikely that an accidental process such as the growing of bacteria into the large bodies would involve all large bodies of a single colony, including the bacterial filaments, and yet never occur in other colonies or in transplants. The most probable supposition to explain the presence of bacteria in the large bodies is that the bacteria were produced within them by transformation of their content.

The bacteria in the large bodies were of about the same size as normal colon bacilli. In some instances they were very small and coccoid in shape and many showed polar staining. In preparations made from the tiny colonies very small polar staining bacilli, round, deeply stained granules and transitional forms are seen frequently.

A previously recorded observation[3] may represent a process similar to that described in this paper. Reference is made to this observation with the purpose of pointing out that the staining properties of the large bodies filled with bacteria were similar to those observed in the present case. In wet preparations the large bodies were deeply stained and conspicuous. In dried preparations their membranes disappeared and their content could not be differentiated from other bacteria.

The observations described are well illustrated in the photomicrographs. From a study of these illustrations it would appear that the bacteria arranged in the shape of the large bodies were formed inside of such bodies. This was clearly visible in the original preparations. Similar observations have been described previously.‡ However, sufficient evidence was never presented to indicate that the forms seen in the large bodies were bacteria, or that the bacteria seen in the preparations originated from the large bodies.

The observation is presented here without further comment. These observations and those described previously suggest that the large bodies are not simply degeneration forms. It can be hoped that by the use of appropriate cultures and technical methods further information can be gained concerning the observed phenomena. The understanding of the nature and significance of the large bodies must await such further studies.

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[3] Dienes, L., *J. Inf. Diseases*, 1939, **65**, 24. (Reference is made to p. 40.)

‡ References may be found in the articles of Klieneberger,[2] Löhnis,[4] Recently Nyberg[5] published similar observations.

[4] Löhnis, F., *National Academy of Science*, Vol. 16, 1921.

[5] Nyberg, C., *Zentralbl. f. Bakt.* (Abt. 1), 1938, **142**, 178; *Acta Path. Microbiol. Scand. Sup.*, 1938, **37**, 401.