OBSERVATIONS UPON THE CYTOLOGY AND LIFE HISTORY OF *FUSIFORMIS*

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(With Plates 4 and 5 and 4 Figures in the Text)

INTRODUCTION

The few studies that have been made of the cytology of *Fusiformis* have been mainly confined to the study of the L cycle of this organism. This paper includes some observations upon the L forms, but it is chiefly concerned with the cytology of *Fusiformis*.

Stable L-forms of *Fusiformis* have been described by Klieneberger-Nobel (1947), who believed that they were symbiotes; but Dienes & Smith (1942, 1943 and 1944) proved that they were a stage in the life cycle of *Fusiformis*, and Klieneberger-Nobel (1949) later agreed with their decision. These workers have confined their studies almost exclusively to those strains of *Fusiformis* which produce more or less stable L-forms that are capable of being subcultured in the L-form stage. The strains to be described all possess a few L-forms which are unstable. All my attempts to subculture these strains in the L-form have failed, because that form invariably reverted to the bacillary form in my cultures.

METHODS AND MATERIAL

All the strains studied were freshly isolated from the human mouth during an investigation of the oral flora. Cultures were maintained, after primary isolation, upon blood-agar plates containing 1 % glucose; they were incubated anaerobically in an atmosphere of 10 % CO_2 at 37° C.

Impression colonies (Klieneberger, 1934) were prepared at intervals of 1 hr. after innoculation of the cultures. These preparations were stained by the tannic acid-gentian violet and the acid-Giemsa methods described by Robinow (1942). Some preparations were stained in a 0·01 % aqueous solution of crystal violet.

OBSERVATIONS

Colony characters. Fusiformis exhibits two types of colony, a smooth colony of 0·5 mm. diameter, and a large, rough, 'medusa head' colony of 2–3 mm. diameter, and these are clearly distinguishable after 48 hr. incubation. Rhizoid types of colonies were also observed. The colonial variants are stable only within certain limits; thus on different occasions a smooth strain may tend towards roughness, and vice versa. The strains so far examined have never, however, undergone a complete and permanent change from one type to another.

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Morphology. The observations recorded here are in agreement with the findings of Spalding & Rettger (1937), who described two groups based upon morphological characters; to wit, group 1, consisting of small organisms averaging 3 by 0·2–0·4μ, and group 2, consisting of organisms averaging 10 by 0·6μ; the former invariably have sharply pointed ends, whereas some of the latter may have blunt ends. To these two groups I would add a third group, consisting of long, thin, wavy, filamentous organisms which vary, in the same culture, from 4μ in length to filaments which extend right across the field of the microscope. Their width also varies, in different strains, from 0·3 to 0·6μ. They may or may not have pointed ends.

There does not, however, appear to be any hard and fast line of demarcation between the groups, and frequently one culture may show organisms of all types, although in most instances one morphological form preponderates.

CYTOLOGY

Cell walls. It was very difficult to obtain clear preparations of *Fusiformis* by the use of the tannic acid-crystal violet technique, due, I believe, to the fact that they have very thin, soft walls. This explanation is supported by the appearance of shadowed electron micrographs of these bacteria, in which the walls have dried so flat that they practically cast no shadow, whereas the very minute particles of debris on the grid cast very long shadows (Pl. 4, fig. 1). The tannic acid-crystal violet staining method did, however, reveal concentrations of stainable material in association with transverse septa at the points of division of the bacilli (Pl. 4, fig. 2). These concentrations of stainable material almost certainly represent the growing points of constituent cells (Bisset, 1951). The presumption that these bands represent the site of the transverse septa was confirmed by the examination of preparations fixed in Schaudin's fixative and stained with a 0·01 % solution of crystal violet, which demonstrated that the cell contents had shrunk and were stained deeply, leaving empty spaces that gave good correlation with the heavily stained bands obtained by the tannic acid-crystal violet technique (Pl. 4, figs. 3, 4). In Pl. 4, fig. 3, the arrow indicates the position of the transverse septa.

Organisms of Slanetz groups 1 and 2 are multicellular and have from 2 to 6 cells per organism (Text-fig. 2). The filamentous organisms of the genus were more easily stained by the tannic acid-crystal violet technique, which revealed them to be multicellular organisms, the individual units of which were much shorter than those of groups 1 and 2. The number of cells per organism was frequently greater than twenty (Text-fig. 1).

Distribution of chromatinic material. This followed the same pattern in all the strains examined. The mature organism has a pair of chromosomes at each pole, and this corresponds to the picture seen in the smooth forms of Eubacteria (Bisset, 1950). The migration and division of these bodies during fission is identical with the process described for Eubacteria, and for *Nocardia* and *Jensenia* (Morris, 1951*a*, 1952). The drawings in Text-fig. 3 show the nuclear bodies as these appear in the filamentous forms, while those in Text-fig. 4 show their distribution in the organisms of groups 1 and 2. The members of the last two groups were often

difficult to stain, and their nuclear material was often included in, and obscured by, a large mass of deeply stained material. However, on occasions it was possible to see that these masses did, in fact, consist of two typical dumbbell-shaped

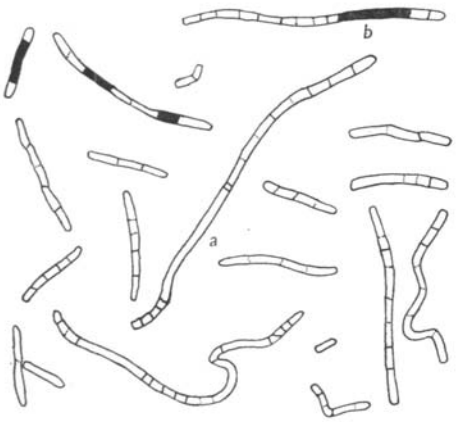

Fig. 1.

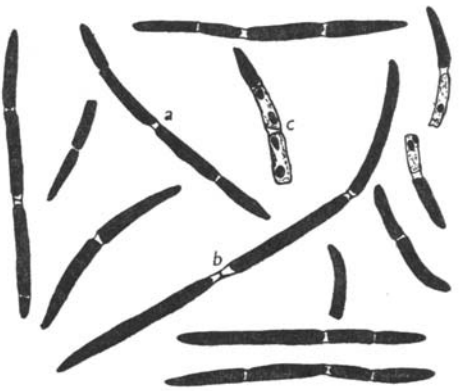

Fig. 2.

Text-fig. 1. The filamentous type of *Fusiformis* stained by the tannic acid-crystal violet method. Showing the small cells in the multicellular organism. *a*, probably represents areas where the stain has failed to show the septa; *b*, represents areas where the entire cell has taken up the stain.

Text-fig. 2. *Fusiformis* corresponding to Slanetz and Rettger groups 1 and 2. Preparations are fixed in Schaudin's fixative and stained by 0·01 % aqueous solution of crystal violet. *a*, showing the empty spaces left by the contraction of the cell contents; *b*, well-formed transverse septa; *c*, damaged cells, but the stained residue of the cell contents correspond to the sites where the nuclear material is usually situated in mature cells.

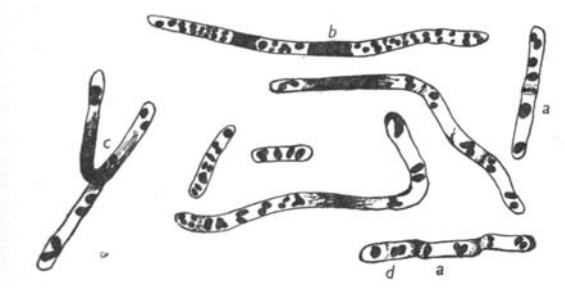

Fig. 3.

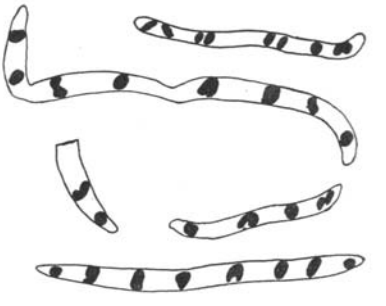

Fig. 4.

Text-fig. 3. The filamentous type of *Fusiformis* stained by acid-Giemsa method. *a*, an organism in which the growing points have also stained, thus showing the distribution of nuclear material within the individual cells; *b*, artefacts due to stain deposits; *c*, false branching appearance due to one organism lying on top of another; *d*, a cell in the state of division.

Text-fig. 4. Organisms as in Text-fig. 2, showing the distribution of the nuclear material.

chromosomes. Electron micrographs of these organisms did not furnish much information; but on occasion shadowed preparations showed granules that were in pairs, and were in the approximate position of the chromosomes (Pl. 4, fig. 5). I interpret these observations as evidence that the deeply stained masses towards the poles of the cells are, in fact, paired chromosomes that have not been resolved.

The L cycle. The L cycle in *Fusiformis* has been noted by Klieneberger-Nobel (1951), by Dienes & Smith (1944), and by Smith, Mudd & Hillier (1948); in all these studies the organisms were specially selected for their ability to throw off L-forms.

During routine investigations of the microbial flora of the mouth, while attempting to prepare impression slides of young colonies of *Fusiformis*, I noticed that occasional organisms were twisted upon themselves in a manner reminiscent of the twisting observed by Klieneberger-Nobel (1951) in the early stages of the *L cycle in the Morax-Axenfeld bacillus. Further study of the Fusiformis strains* which were subsequently isolated, showed that a few organisms in every strain, when freshly isolated, passed through the L cycle. So far, thirty-eight strains have been examined which have exhibited this feature.

The organism described by Wherry & Oliver (1916) under the name *Leptothrix innominata* was undoubtedly *Fusiformis* of the type group 2. These workers show some very fine drawings of this organism twisting upon itself, and they also have an illustration of this organism showing swollen forms similar to those which are described later in this paper.

The first changes were observed from 2 to 4 hr. after subculture, and in the strains examined there would appear to be three ways of initiating the L cycle. The organism may twist upon itself, as in the Morax-Axenfeld bacillus (Pl. 5, figs. 7, 8); or it may form loops, as described by Klieneberger-Nobel (1951) in *Proteus* (Pl. 5, figs. 6, 10); or it may fold back upon itself (Pl. 5, fig. 9). When looping occurs, a large swelling appears at the junction of the loop (Pl. 5, figs. 10, 11). If the organism folds back upon itself, a large body generally appears at the end of the filament, and, in the early stages of this type of development, two dense masses of chromatinic material usually appear in the swelling (Pl. 5, fig. 12). These chromatinic masses then appear to fuse and, at the same time, the filament seems to lose its regular outline and to become shorter, while the large body expands (Pl. 5, fig. 13).

Further development takes place by one or other of the following ways. The large body may disintegrate and liberate minute amorphous granules (Pl. 5, fig. 14), or it may continue to swell until it occupies the whole of the original filament. Eventually these swollen forms thrust out finger-like protrusions (Pl. 5, fig. 15).

The final phases are the same in both instances, namely, the production of a mass of small particles. The protrusions of the second form become separated from the parent mass and divide by segmentation to produce a mass of small particles, whereas the amorphous mass of the first form becomes granular and eventually is transformed into a mass of small particles. Such masses multiply rapidly until the cultures are from 4 to 10 hr. old, after which each single particle appears to pass through a distinct morphological change to produce a young, normal *Fusiformis* (Pl. 5, figs. 17, 18). Branching occurs during this period.

The usual methods employed in the cultivation of L-forms have all failed to produce a stable L-form in any of the strains examined.

DISCUSSION

Fusiformis is a multicellular organism which has the cytological characteristics of the Eubacteria, and its usual manner of reproduction is by simple fission. In all freshly isolated strains there exist a few cells which possess the ability to reproduce by means of the L cycle; but this appears to be only a temporary phase in the life history of the organism, and these L-forms cannot be cultivated in a stable form. In my cultures, mixed plates from mouth washings frequently produced minute hazy colonies, which were found to be composed of irregular amorphous granules which could not, however, be subcultured. It is impossible to determine in such mixed cultures the exact nature of these colonies; but, since none of the other organisms isolated from the mouth has shown any tendency to produce L-forms, I believe that these colonies may be L-forms of *Fusiformis*. All strains examined lost the ability to reproduce by the L cycle after repeated subculture. The earliest loss occurred after the fourth subculture, whereas one strain continued to reproduce in this manner until the twenty-third subculture. Klieneberger-Nobel (1952) considers that bacteria revert to the L-form under adverse conditions, but I consider that with *Fusiformis* the L cycle is a normal function of the organism under normal conditions.

The mode of formation of the large bodies in the strains examined in this study differs notably from that described by Dienes & Smith (1944) and Smith *et al.* (1948). There are *Fusiformis* which produce the large bodies in a manner described by these workers and have a stable L-form; but there are also *Fusiformis* which pass through the L cycle in the manner described in this paper. The former are only occasionally observed, whereas the latter occur in all freshly isolated strains. In Dienes's strains, the initial stage of the L cycle appears to be a swelling of the adjacent cells of the organism, and this stage is probably followed by a conjugation of the two cells. This initial process is analogous to the conjugation of adjacent cells observed by Morris (1951 *b*) in *Actinomyces*, where two haploid cells fuse to form the diploid form from which the secondary phase arises.

I believe that, however the large bodies may be initiated, the process represents some form of sexual conjugation as has been suggested by both Dienes & Smith (1944) and Klieneberger-Nobel (1951).

SUMMARY

Fusiformis is a multicellular organism having the cytological characters of the Eubacteria, and normally reproducing by fission.

A few cells of all the strains examined pass through the L cycle; usually the initiation of this process follows the patterns described in *Proteus* and the Morax-Axenfeld bacillus. It has not so far been possible to grow these L-forms of *Fusiformis* in culture without immediate reversion to the bacillary form.

The stable forms described by other workers appear to be produced by a process analogous to the sexual conjugation seen in the *Actinomyces*.

The evidence suggests that passage through the L cycle is a normal function of *Fusiformis* in its natural habitat.

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EXPLANATION OF PLATES 4 AND 5

PLATE 4

Fig. 1. Electron micrograph of *Fusiformis*. The flatness of the organism is shown by comparison of the shadows of the organism and those of the debris. × 8000.
Fig. 2. *Fusiformis* stained with tannic acid-crystal violet. The bands of stained protein matter mark the site of the transverse septa. × 3000.
Fig. 3. *Fusiformis*, Schaudin fixed and stained with 0·01 % crystal violet showing the contraction of the cell contents, leaving empty spaces at the sites of the transverse septa. The arrow indicates a well-formed septum. × 3000.
Fig. 4. As fig. 3, but fixed in methyl alcohol.
Fig. 5. Electron micrograph of *Fusiformis*, showing the tendency of the granules to be paired. × 8000.

PLATE 5

Fig. 6. *Fusiformis* showing the looping process before the formation of L bodies. × 1500.
Figs. 7, 8. *Fusiformis* showing the twisting process before the formation of L bodies. × 1500.
Fig. 9. *Fusiformis* showing the organism folding back upon itself before the formation of L bodies. × 1500.
Fig. 10. *Fusiformis* showing an organism forming a loop, and another showing a swelling starting where the loop has closed. × 1500.
Fig. 11. *Fusiformis* showing a swelling where a loop has closed. × 1500.
Fig. 12. *Fusiformis* showing a swelling at the tip of the organism. This is the result of the organism folding back upon itself. Two darkly stained bodies are seen inside the swelling. × 1500.
Fig. 13. *Fusiformis* showing the enlargement of the swelling and shortening of the cell. × 1500.
Fig. 14. *Fusiformis* showing a terminal swelling that has ruptured, freeing an amorphous mass.
Fig. 15. A large body derived from *Fusiformis* showing finger-like protrusions. × 1500.
Fig. 16. A mass of L bodies.
Fig. 17. A mass of L bodies which are reverting to normal fusiform state. The arrow shows a young bacillus forming. × 1500.
Fig. 18. As fig. 17. Shows branching during the organization of the granular mass into bacilli. × 1500.

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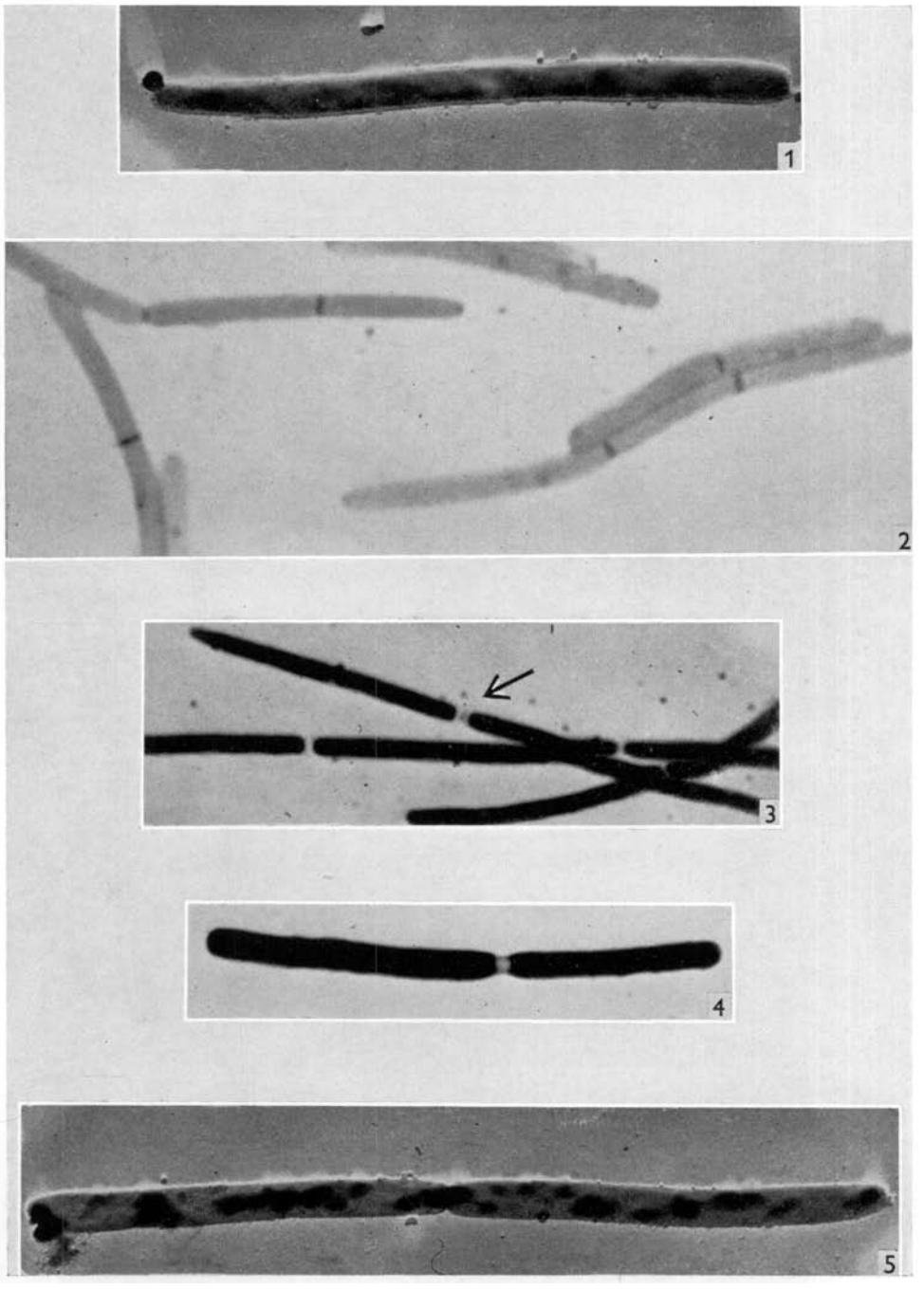

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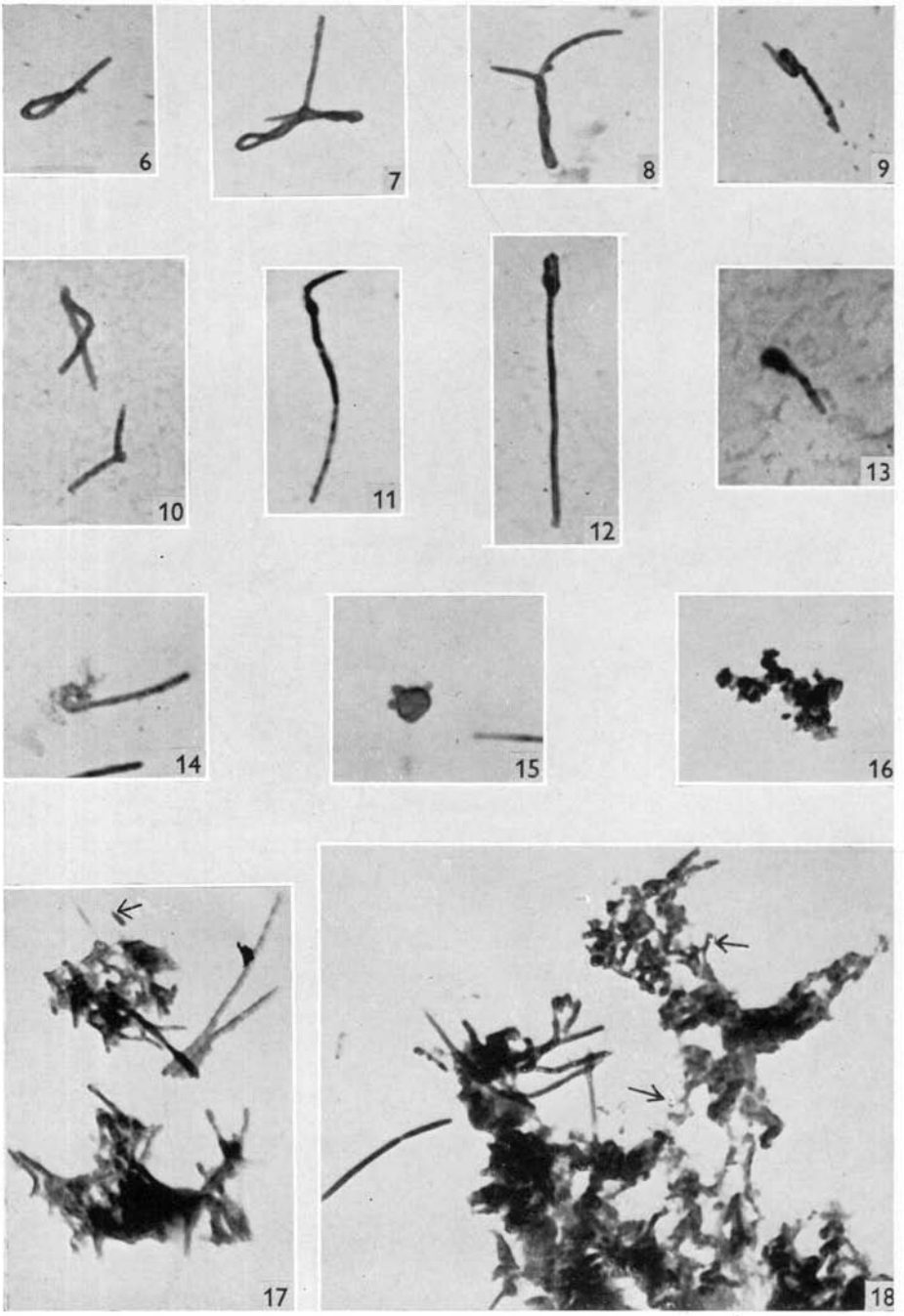

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