MORPHOLOGICAL APPEARANCES OF VARIOUS STAGES IN *B. PROTEUS* AND *COLI*

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(With Plate 7)

The well-known zonal swarming of *B. proteus* on solid media is produced by the development of long filamentous bacterial forms which alternate with short forms as described by Russ-Münzer (1934–5). When a certain area has been thinly covered by the 'swarmers' they stop their movement and divide into short almost immobile bacteria which multiply to form a thick layer. Then 'swarmers' again develop to cover a new area. The whole cycle may be completed in approximately 4 hr. It seemed of interest to apply newer staining methods by which chromatinic structures are being demonstrated in order to find out if some change in the chromatinic apparatus is involved in this phenomenon.

A well-swarming *B. proteus* culture 'E' was thinly inoculated on nutrient agar plates. Small pieces were cut out of the plates, placed on sterile coverslips, the inoculated side next to the glass surface, and the microcultures were incubated in a moist chamber. After various periods of time the agar pieces were peeled off and the preparations fixed and stained as described in previous papers (Klieneberger-Nobel, 1945, 1947*a*, *b*; see also Robinow, 1945). In addition, impression preparations were taken from 'swarming plates' at different stages of growth. The development followed the same course on media of different constitution and differing pH, though it was speeded up on some media and slowed down on others. For most observations a trypsin digested meat infusion agar of pH = 6·8 was used. The course of events was also followed up in preparations from broth cultures fixed and stained in a similar way. Apart from differences in the length of the filaments the development followed the same trend under all the conditions studied in *B. proteus* as well as in *B. coli*. Therefore the following description applies to both organisms.

The transformation during the lag phase and the appearance of the very young organisms have been clearly demonstrated by Robinow (1945), but the development has not been followed up further by him. After 2 hr. of incubation the culture consists of fairly wide cells containing two or four fat and conspicuous, dividing 'chromosomes' as shown in Pl. 7, fig. 1 (see also Robinow (1945), diagram, p. 358, under O, H, I and his Figs. 12 and 13, Pl. II). The relatively large size of these organisms is very obvious from the colloidal silver preparation as illustrated in Pl. 7, fig. 3. It was prepared from a 2½ hr. old broth culture of *B. coli* '4C'. After the third hour of incubation (the time varies with the amount of inoculum, the quality and reaction of the medium and the temperature of incubation) the whole growth undergoes a drastic change. The large cells with their four conspicuous 'chromosomes' no longer show such an orderly arrangement of their chromatinic structures, as can be seen from the large cells in Pl. 7, figs. 2 and 5, and divide up quickly into very small cells. The four-cell filament representing the typical young cell (four 'chromosomes') may first divide into a two-cell filament (two 'chromosomes'), and eventually it will divide into the smallest possible unit, a cell with one nuclear body only. The various kinds of cells can be seen in Pl. 7, figs. 2 and 5. The differences in the sizes of the cells are so great that the unbiased onlooker may doubt the purity of the culture from which the preparation was made. Yet all the cells are elements of one pure culture. Very soon the large cells disappear completely, and only the small cells into which they have divided are found. These small cells are very motile and swirl about in a drop of liquid. They soon produce thin filaments composed of two to four small cell units. A colloidal silver preparation of the small-cell stage is illustrated in Pl. 7, fig. 4. While in *B. coli* the filaments remain short on both liquid and solid media in *B. proteus* they grow out into very long filaments, but only when it is grown on solid media. These long filaments are the well-known 'swarming' elements. When they are stained for chromatinic structures it can be seen that they are narrower than the 2–3 hr. cells, and that their 'chromosomes' seem somewhat shorter than those of the young four-cell filaments (cp. Pl. 7, fig. 1 with fig. 6). When the 'swarmers' are fixed through the agar with Bouin's solution and stained slightly with Giemsa (Robinow, 1945, p. 374), the cytoplasm of the cells is stained almost exclusively.

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It will then be seen, as illustrated in Pl. 7, fig. 9, that the length of the filament is severed into small compartments by transverse protoplasmatic layers. Between these layers, which appear as bands in the optical section, empty spaces are noted, in which the chromatinic structures, demonstrable only by the hydrochloric acid-Giemsa method (or Feulgen's method respectively) are situated. In the middle layer of these cytoplasmic formations transverse cell walls may develop which may eventually separate the compartments from their neighbours so that the filaments will divide into small cells. These facts go to show that the 'swarmers' of *B. proteus*, and the small cells of both *B. proteus* and *coli*, represent a similar stage in the two organisms.

The habit pictures of the young large-cell type and of the older small-cell type are so different that it was thought at first that this difference might be due to changes in the chromatinic matter possibly involving a kind of reduction division; but it can be shown that their varied appearances are entirely ruled by environmental circumstances. When the culture is in the small-cell stage, no matter what its age, the small cells transform themselves into large cells as soon as they are transferred to fresh media. On the other hand, large forms transplanted on a new medium go on to produce large daughter cells until such conditions have been produced which will induce them to divide up into the small-cell generation. According to Lominski & Lendrum (1947) swarming is controlled by metabolites. Presumably, metabolites must also be responsible for the subdivision of the large cells into the small-cell generation, representing the rudiments of the 'swarmers'.

Thus we are in a position to explain the peculiar phenomenon of the zonal swarming of *B. proteus* on agar plates. Whatever the age of the material inoculated on to the middle of a fresh agar plate, a generation of large cells is produced in the area of inoculation. When by the production of metabolites conditions have arisen which stimulate the large cells to subdivide, small motile cells are produced which multiply rapidly and grow out into long aggregates, the 'swarmers', and cover by their movements a circular zone round the place of inoculation, the area of which depends on the concentration of the agar. At the end-point of diffusion where the concentration of metabolites has fallen off to zero, both the swarming and multiplication of the small-cell generation come to a standstill, for now they behave in the same way as if transplanted to new media. They transform themselves into the young large cells and produce new generations of large daughter cells until the increase in metabolites has reached the point that stimulates again their subdivision into the minimal cellular units. As soon as the small cells have formed, swarming commences again. Therefore, when impression preparations are made from the edges of the growth of 'swarming plates' it will be found that sometimes 'swarmers' build up the edge, sometimes large young cells and sometimes (but more rarely because this stage is very transitory) a uniform mass of tiny cells is present. It is also obvious that on a plate into which a filtrate of a *proteus* culture has been incorporated (an experiment performed by Lominski & Lendrum) an inoculum will immediately develop into the small-cell stage and will move over the whole plate without halting, because the large young cell generation develops only on fresh nutrient media.

As 'fusion' cells have been observed in various organisms studied by the writer, such as spore-bearing bacilli, *Actinomycetes* and *Myxococci*, a search for 'fusion cells' was also undertaken in *B. coli* and *proteus*. In most bacterial cultures isolated cells are found in which the nuclear material forms one continuous, rod-like body, but areas in which cells are thus transformed uniformly do not occur. Yet when young cultures are stored in the cold or left on the bench at a cold room temperature they often show fields in which most of the organisms appear as illustrated in Pl. 7, figs. 7 and 8. The chromatinic material of each compartment has concentrated into a central rod varying in length with the compartment. Such a contraction of the chromatinic material has already been observed to take place regularly in young vegetative cells of spore-bearing anaerobes as soon as they are exposed to air (Klieneberger-Nobel, 1945), and it may be suggested that this apparently most important chromatinic material contracts and draws back from the cell walls into the interior as a protective measure against unsuitable conditions. It may be of interest to investigate further these relatively rapid reactions of a presumably very sensitive material.

SUMMARY

It has been shown that in all cultures of *B. proteus* and *B. coli* a large-cell type with two to four 'chromosomes' develops first and produces, after a few hours of growth, by sudden subdivision the smallest unit, a generation of small cells, each with one nuclear structure only. This cell type, singly or in filaments, persists until the end of the growing period. On a 'swarming plate' of *B. proteus* a number of these biphasic growing periods may follow each other.

'Fusion cells', in which the chromatinic structures have contracted into a longitudinally arranged bar, are formed when young cultures are stored in the cold.

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EXPLANATION OF PLATE 7

(Magn. 1 : 3000, approximately)

Fig. 1. *B. coli* stained for chromatinic structures, 2½ hr. growth, large-cell type.
Fig. 2. *B. proteus* stained for chromatinic structures, 3 hr. growth, large-cell type on the verge of subdivision. A group of small cells is already to be seen.
Fig. 3. *B. coli*, colloidal silver preparation, 2½ hr. growth, large-cell type.
Fig. 4. *B. coli*, colloidal silver preparation, 4 hr. growth, small-cell type.
Fig. 5. *B. proteus* stained for chromatinic structures, 3 hr. growth, stage of subdivision, a few large cells are still present together with many two-cell and one-cell units; note the smallness of the newly formed one-cell unit.
Fig. 6. *B. proteus* stained for chromatinic structures, field containing 'swarmers' only. Compare with fig. 9.
Fig. 7. *B. proteus* stained for chromatinic structures, from a 'swarming plate' which had been stored in the cold. Note the concentration of the nuclear material.
Fig. 8. *B. proteus* stained for chromatinic structures, from a plate which had been standing at cold-room temperature. Note 'swarmers' and small cells with chromatinic 'fusion bodies'.
Fig. 9. *B. proteus* stained for cytoplasm. Note transverse bands and cytoplasmic layer at both tips of the filament. Compare with fig. 6.

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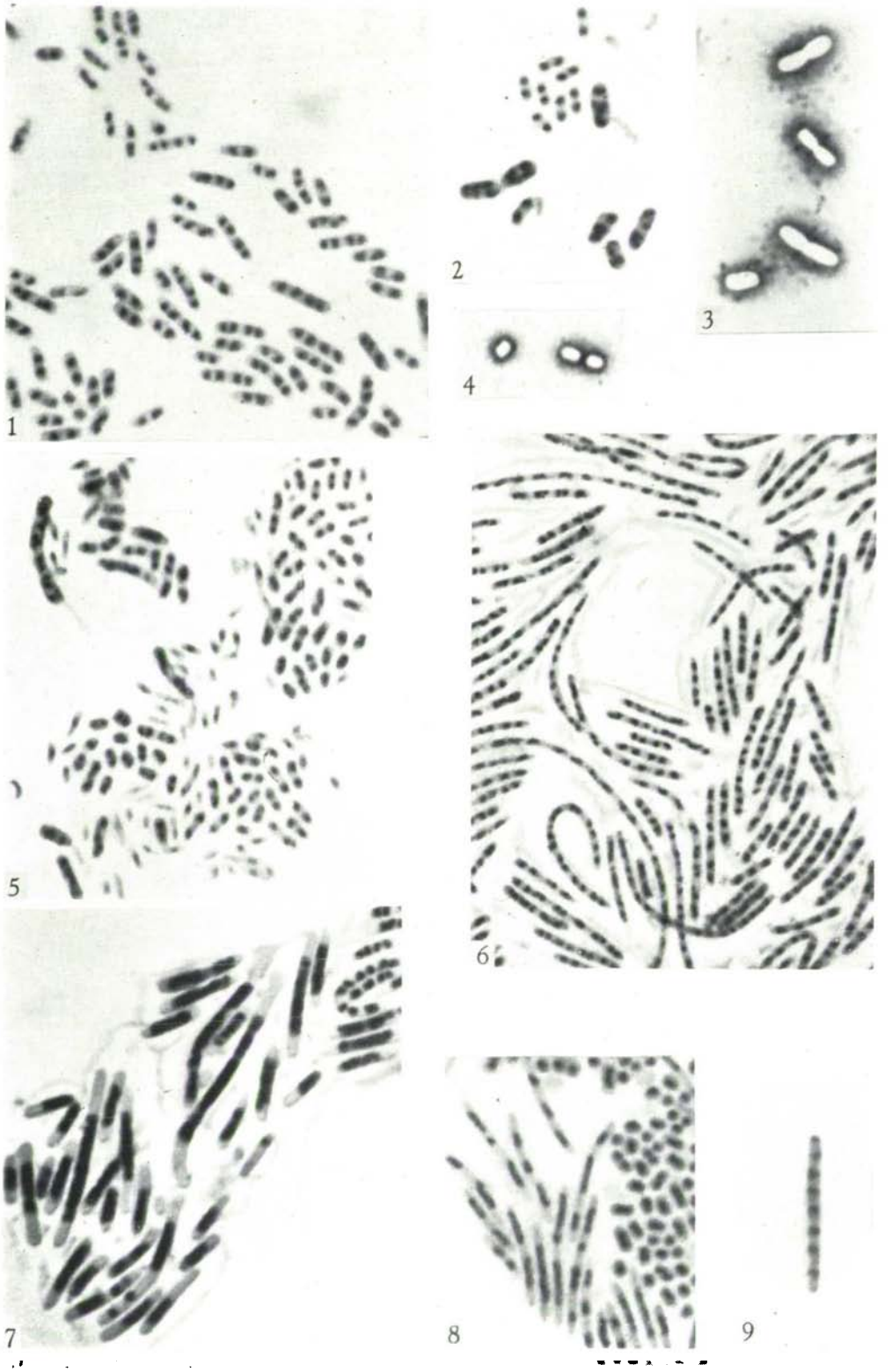

Figs. 1—9

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