CHANGES IN THE NUCLEAR STRUCTURE OF BACTERIA, PARTICULARLY DURING SPORE FORMATION

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(With Plates 2–4, and 3 Figures in the Text)

INTRODUCTION

Confirming and extending the basic observations of Badian (1933–6), Stille (1937), Piekarski (1937–40) and Neumann (1941), Robinow (1942) has shown in his paper on the nuclear apparatus of bacteria that 'the structural unit of the nuclear apparatus of aerobic, spore-forming bacteria is a dumbbell-shaped body, giving a positive Feulgen reaction and possessing a strong affinity for nuclear dyes'. According to him bodies of this kind, one of which is contained in the resting spore, play an essential part in cell division and spore germination. They divide longitudinally and their division precedes the division of the vegetative cells. For these reasons Robinow compares them to the chromosomes of plant and animal cells. Clear pictures of chromatinic dumbbell bodies, 'chromosomes', have also been demonstrated in Feulgen-stained preparations by Neumann (1941); he examined *Bacterium proteus*, *Bact. pyocyaneum*, *Bact. coli*, *Bacillus mycoides* and *B. anthracis*. Later, a large number of bacteria belonging to different groups of organisms, such as spore-bearing aerobes and anaerobes, members of the coli-typhoid group, *Bacterium shigae*, *Bact. pestis*, staphylococci, streptococci, sarcinae, *Corynebacterium diphtheriae*, *Mycobacterium tuberculosis*, spirilla, actinomyces and others, were examined by Robinow's method,* and all of them showed a similar chromatinic apparatus which takes the form of one dumbbell body in each cell unit (Lewis, 1942; Robinow, 1944).

The observations recorded in this paper indicate that the nuclear apparatus plays an essential part in spore formation, for in both aerobic and anaerobic spore-bearers, characteristic and similar configurations of the chromatin material occur which are strongly suggestive of a nuclear fusion followed by a reduction of the chromatin substance during sporulation. Secondly, a nuclear fusion resembling the process observed in spore formation but set in motion by a special stimulus will be described in anaerobic sporulating bacilli. Lastly, an account will be given of the nuclear apparatus of *Sphaerotilus natans*, a non-sporulating water organism, and the fusion of chromatinic material in the older cells of its growth.

* This statement is based on a private communication by Dr Robinow, on the results of my own work and on my study of the literature of the subject.

THE MICRO-ORGANISMS CHOSEN FOR STUDY

These were *Clostridium welchii*, strain 'A 118'; *Cl. septicum*, strain 'K.F.'; *Cl. oedematiens* var. *gigas*,* strains 'Albiston', 'Tongala', and 'gigas 1'; *Bacillus mycoides*, strain '926, N.C.T.C.'; and *Sphaerotilus natans* Kützing* (see P. Linde, 1913; H. Zikes, 1915).

METHODS

3% peptone meat-infusion agar was used as a basic medium for the anaerobic organisms, surface plate cultures of which were incubated in a McIntosh-Fildes jar. On this medium *Clostridium welchii*, 'A 118', formed spores freely in 2–3 days. On the basic medium enriched with 10% horse serum *Cl. septicum* formed an abundant mass of spores in 1–2 days. The '*gigas*' strains failed to grow on a surface plate unless 3 c.c. of a suspension of fresh-ground brain substance in horse serum was added to the basic medium. On this mixture they grew well and formed spores after 2 or 3 days' incubation. *Sphaerotilus natans* was cultivated on the following medium: glucose 0·2%, meat extract 0·1%, agar 1·5%; *p*H 7·0.

The organisms were fixed and stained according to Piekarski (1937) and Robinow (1942). Wet-impression preparations on coverslips, as far as possible consisting of one layer of cells only, were fixed in osmic acid vapour for 3–5 min. After drying they were immersed in normal hydrochloric acid for about 9 min. at a temperature of 53–55° and were then washed and stained in 1 : 20 Giemsa solution for 10–30 or 60 min., according to the staining properties of the specimen. They were dehydrated in mixtures of acetone and xylol and mounted in Canada balsam. The preparations were

* I am indebted to Dr J. Keppie for a number of strains of *Clostridium oedematiens* var. *gigas* and to Prof. E. Pringsheim for several cultures of *Sphaerotilus natans*.

studied and photographed under optical illumination by means of the following lens system: Zeiss achromatic condensor, n.a. 1·4; Zeiss apochromatic oil-immersion $\frac{1}{12}$, n.a. 1·4; Zeiss compensation ocular × 15; the magnification thus produced was × 1350. The preparations were mounted in the diluted Giemsa stain or in water, and a preliminary examination was made before the final dehydration and differentiation was carried out.

It should be emphasized that, in order to avoid changes due to air exposure—as will be described later—all preparations of anaerobes used for the study of the nuclear apparatus were made from material fixed immediately after opening the anaerobic jar.

THE NUCLEAR CYCLE IN SPORE FORMATION

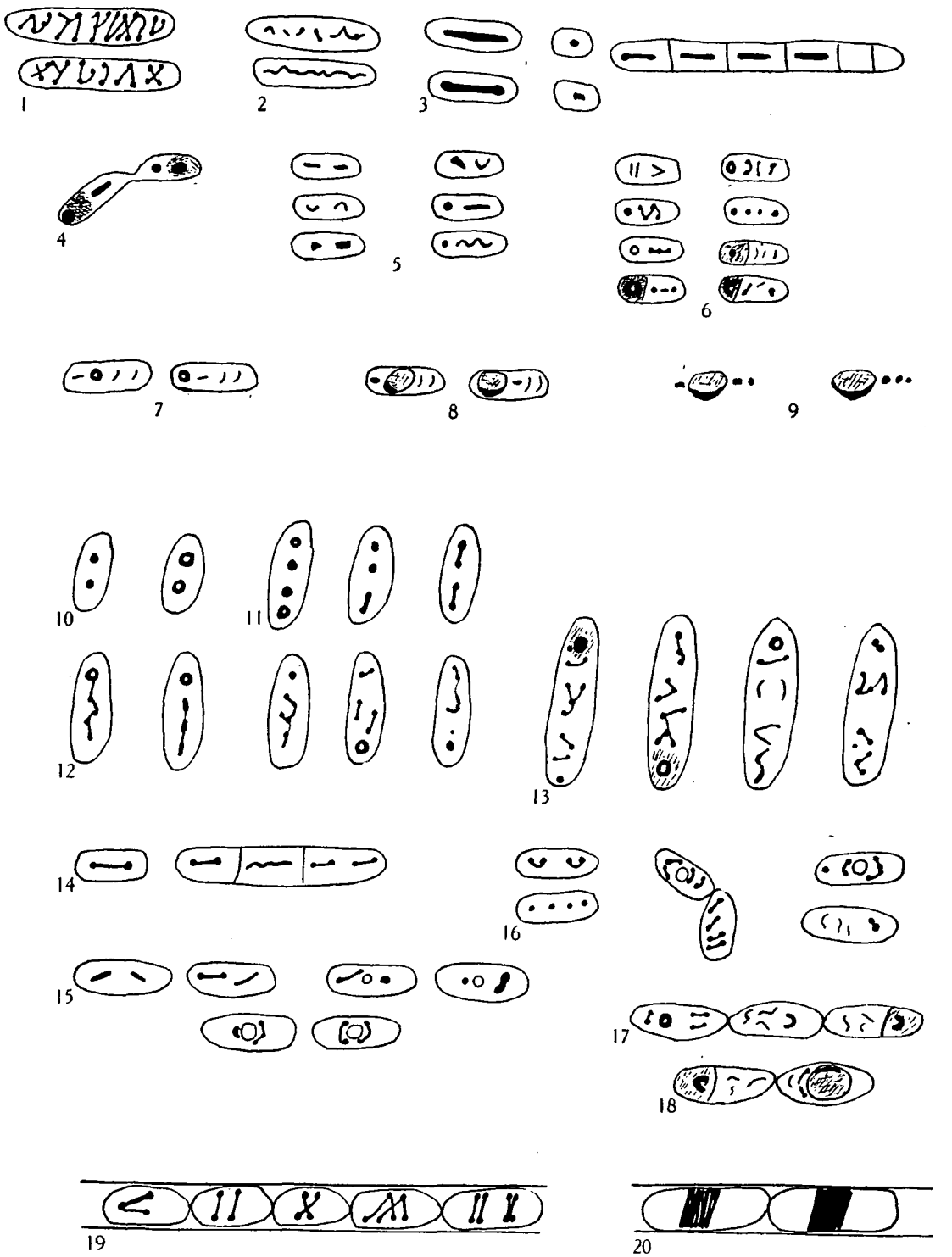
The behaviour of the nuclear apparatus during spore development will be described separately for the four species studied, as they vary in detail though not in their general features.

Clostridium welchii. If inocula from fairly young cultures are used, rapid growth is obtained within a few hours of incubation. When, after about 5 hr., the first examination of the cultures was made the young elements, then present in abundance, consisted of more or less long cells containing a number of deeply staining nuclear structures, all of them arranged more or less parallel to the short axis of the cell, but otherwise irregularly distributed; the dumbbell shape of these 'chromosomes' was often recognized. The young filamentous cells, as described, are illustrated in Text-fig. I, 1, and Pl. 2, phot. 1. As each of them contains up to many pairs of dumbbell bodies, they represent multinuclear organisms. The occurrence of elements of large size with multiple chromatinic structures has been described by Smiles and myself for pleuropneumonia-like organisms (1942) and for the *L*1 organism in 1942 by myself.

With increasing age of the culture the filamentous forms of *Cl. welchii* change into shorter but still elongated and multinuclear elements. At the same time the nuclear structures referred to as 'chromosomes' undergo a remarkable change. They assemble along the long axis of the cell, contract, becoming stouter and shorter, and eventually join to form a compact, deeply staining cylinder placed in the middle of the cell parallel to its longer axis (Text-fig. I, figs. 2, 3). This mode of nuclear fusion may perhaps be regarded as a kind of autogamic process, which changes the appearance of the cells completely, as is evident if Pl. 2, phot. 1 (5 hr. growth) is compared with Pl. 2, phot. 3 (12 hr. growth); the fusion cells are more uniform than the 'chromosome' stage, they are shorter and contain axial chromatinic rods. Occasionally fusion cells are very short and enclose an almost spherical fusion body. They may also be linked up together in fairly long chains and are then segregated from each other by well-stained septa; Text-fig. I, 3 shows a variety of fusion cells in *Cl. welchii*. It may be mentioned at this point that spores which form in the further course of development always originate from fusion cells and are never produced from cells with the transverse dumbbell structures: nuclear fusion precedes spore formation. The question arises here: Are fusion cells—unless their development ceases altogether—bound to form spores or are they able to divide into daughter fusion cells? This problem has not yet been fully investigated, but it may be noted that, when fusion cells were transferred to a fresh nutrient medium, they seemed to be transformed into the young growth.

Pl. 2, phot. 3, shows fusion cells of *Cl. welchii*, most of which are of approximately the same size; their cytoplasm was stained a delicate blue, and the central nuclear cylinder a deep red, thus proving that the cells in question were in an active state of development; and in fact most of them can be regarded as spore mother cells. In the left-hand bottom corner of phot. 3 a couple of cells are seen that have developed further than the surrounding spore mother cells; in this particular pair the nuclear cylinder of each cell has been halved. One of the partition products is to be seen in the innermost part of the cells, which in the original preparation were lightly stained; the other half is seen in the more darkly stained outermost part of both cells, and these will develop into spores as indicated by their deeply staining cytoplasm. The drawing, Text-fig. I, 4, demonstrates, perhaps even more clearly than the photograph, the nuclear apparatus and the contrasting cytoplasmic parts of the spore mother cells in question. As shown in this example the first division step of the axial nuclear cylinder of the spore mother cell produces usually two nuclear bodies, each of which contains one-half of the original material; but sometimes a segregation of an element representing only one-quarter of the material takes place although the equivalent of the remaining three-quarters has not yet divided. Some of the most common configurations of nuclear material produced by the first division of the chromatin cylinder are shown in the drawings (Text-fig. I, 5).

When the second division of the nuclear material of the spore mother cell takes place, the dumbbell bodies or 'chromosomes' may appear again. Text-fig. I, 6, illustrates configurations found after the second division. From these observations it would appear that the fusion body of the spore mother cell is equivalent to four 'chromosomes' which, when spores are formed, are usually brought to



Text-fig. 1. Showing the behaviour of the nuclear apparatus during spore formation in *Clostridium welchii*, *Cl. septicum*, *Cl. oedematiens* var. *gigas*, *Bacillus mycoides* and in vegetative cells of *Sphaerotilus natans*.

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light as the result of two division steps closely following each other. As can be seen from the drawings, one of these four 'chromosomes' has more often the shape of a small round body than the dumbbell form, or it may be crescent- (spheres?), loop- or circle-shaped. Chromatin rings and crescents have already been described in the resting and germinating spore by Robinow (1942). This particular structure usually becomes the spore 'chromosome'; in the course of development it swells up and takes the stain more deeply; often a membrane can be demonstrated which has formed to separate the spore 'chromosome' with its surrounding cytoplasm from the remaining part of the spore mother cell. This remainder gradually decays after the spore part has undergone its final development and has become surrounded by the spore membrane. The last three drawings of Text-fig. I, 6, show some of the configurations obtained when the differentiation of the spore part and the remaining part of the mother cell has taken place. Pl. 2, phot. 4, illustrates a number of spore mother cells after their second nuclear division. Dumbbell forms are found in some of the cells, in some of which the swollen spore 'chromosome' is clearly visible. Some organisms show more than four structures. This may be due to various causes: for example, one dumbbell may show as two granules, the connecting rod not being stained; or the structures that are destined to be eliminated may have disintegrated; moreover, the three 'chromosomes' that are of no further consequence to the cell will not always segregate completely and are often connected by chromatinic strands which may be taken for 'chromosomes'; and further, as is seen in the photograph, a few cells are larger than the ordinary spore mother cell and these probably represent more than one cell. Nevertheless, the conclusion seems justified from many isolated observations that one part of the nuclear apparatus of the spore mother cell develops into the spore 'chromosome' and that the rest, representing three-quarters of the nuclear substance, perishes. When the spore is completed, the nuclear apparatus of the spore mother cell is the last thing to disappear, and thus remains of this material are often to be found either on one side or on both sides of the mature spore at a time when the walls and cytoplasm of the mother cell have already disappeared. The position of the nuclear debris must obviously depend on the original position of the spore 'chromosome' in the spore mother cell. There are two possibilities and the configurations resulting from them are illustrated diagrammatically in the drawings (Text-fig. I, 7–9).* Naturally the set of nuclear granules attached to the young spore is often incomplete, so that usually only one or two granules or little rods are found attached to the spore instead of three.

When the spores are formed quickly and the spore mother cells are relatively small, the two division steps described cannot always be clearly recognized. The consecutive stages can be only properly observed when the cells are of relatively large size, when they are flattened out on the cover-slip and when the fixation, staining and differentiation are adequate.

Clostridium septicum. The nuclear fusion is similar to that seen in *Cl. welchii*. The fusion cells are usually arranged in long chains; the single elements are slender, and their cytoplasm stains faintly, but they contain well-stained, often club- or dumbbell-shaped fusion rods. The study of the reduction division involves great difficulties owing to the narrowness of the cells, but as a rule two stages can be demonstrated with clearness. The spore mother cells, which are often arranged in couples, become very transparent after the first nuclear division; the intensification of the cytoplasm of the spore half of each mother cell is then very marked. The stage of segregation of the four nuclear structures cannot be seen so clearly as in *Cl. welchii*; but when the young spore is almost completed it can frequently be observed that a division of the nuclear rod has taken place; three of the structures are then eliminated and one remains to form the spore as shown in Pl. 4, phot. 18. When preparations of *Cl. septicum* are made from sporing cultures, chromatinic tags corresponding to eliminated 'chromosomes' can, as a rule, be demonstrated. This is illustrated in Pl. 4, phot. 14, in which some spores show nuclear granules or rods on one side only, and others show them on both sides.

Clostridium oedematiens var. *gigas*. This large bacillus is very suitable for cytological studies. Very young cultures have not been examined, but after about 8–12 hr. incubation growth was often well established on the brain suspension plates, and preparations fit for cytological examination were obtained. This growth phase is very filamentous and the single elements vary in length. Fusion may start after 24 hr. incubation, when gradually more and more cells are being transformed. The fusion cell is wide and transparent; the cell boundaries are well defined; the cytoplasm stains light blue; the chromatinic cylinder deep red and often almost black. Pl. 3, phots. 9, 10, show typical young cells with transverse dumbbell bodies. Two fusion cells are to be seen in *a* (phot. 10). The nuclear apparatus in most of the cells included in phot. 9 is seen at the beginning of the process of fusion; in the middle section a fusion cell contains four fusion bodies.

In about 2 days most cells of a well-grown surface

* It should be mentioned here that Badian in 1933 had already pointed out these two possibilities.

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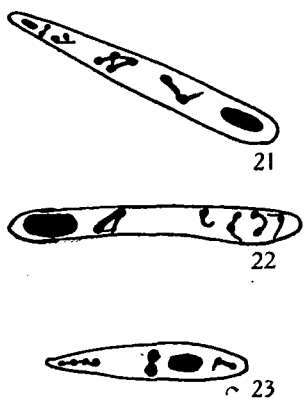
culture have developed into fusion forms. Some have gone farther than others and are forming spores; but abundant spore formation was not often observed. Many spore-forming cells are relatively long and correspond to two spore mother cells; these may produce two spores, one at either end; short single-spore mother cells were also found. The first step in the development is a division of the fusion cylinder into two or four fairly big, darkly staining roundish bodies, two in the single-, and apparently four in the double-spore mother cell (Pl. 3, phot. 9); these bodies sometimes appear ring-shaped. Occasionally only one or two round bodies are segregated from the remaining chromatin rod. The various configurations of the chromatin material met with during the first nuclear division in single- or double-spore mother cells of *Cl. oedematiens* var. *gigas* are illustrated in Text-fig. I, 10 (single), 11 (double-spore mother cells).

After the next division the round-spore 'chromosome' is usually to be found at the end of the cell. If the organism represents two-spore mother cells, one of them shows, as a rule, an advanced development as compared with its neighbour. The drawings, Text-fig. I, 12, shows single- and Text-fig. I, 13, double-spore mother cells after the second division. The single-spore mother cell should contain 4, and the double 8 nuclear structures; it seems that often not all the superfluous 'chromosomes' that will be eliminated are disconnected, so that they remain joined together by chromatin strands until the spore is ripe. Pl. 4, phots. 15, 16, both show one double-spore mother cell; the corresponding drawings, Text-fig. II, 21, 22, demonstrate how their nuclear configurations can be interpreted. Pl. 4, phot. 17 (compare Text-fig. II, 23), shows a single-spore mother cell with the young spore; the three structures to be eliminated are clearly visible.

Bacillus mycoides. This was the only aerobic spore-bearing organism studied. The behaviour of the chromatinic material during spore germination and during the early growth of the young culture has been clearly described by Robinow (1942). When I started to study spore formation in *B. mycoides* preparations were taken from potato agar plates, on which spores are formed rapidly. Owing to the great density of the spore mother cells it was at the time not possible to obtain sufficiently transparent specimens to allow study of the internal structures. It was then learned from a paper of Holzmüller published in 1909 that *B. mycoides* will not form spores in deep broth tubes where it grows as a sediment, but will do so when the bacterial filaments are transferred to distilled water, provided that there is access of air. As it was thought that spore mother cells developing slowly in distilled water might be transparent, the method of Holzmüller was adopted. The organism was grown in broth tubes for 2–4 days. The tubes were centrifuged, and the sediment was washed once in distilled water and then transferred to a shallow layer of distilled water. Droplets were taken out at intervals and placed on cover-slips which were treated as described.

After about 2 days in distilled water the chromatin structures in many of the cells stained very faintly. In others in which nuclear fusion was taking place or had been accomplished the chromatinic material stained deeply. These fusion cells completed sporulation on the third day and provided suitable cytological material, as illustrated in Text-fig. I, 14–18. When the fusion cells undergo their first nuclear division, the chromatin may be divided either in the proportion 2 : 2 or in the proportion 1 : 3 as shown in Text-fig. I, 15. The next division step sets free the four nuclear structures enclosed in the fusion cylinder. At this stage the mother cell frequently contains a vacuole



Text-fig. II. Drawing corresponding to Pl. 4, phots. 15–17, showing how the nuclear configurations can be interpreted.

round which the nuclear structures have arranged themselves (Text-fig. I, 15, 16). The spore 'chromosome' usually takes the shape of an open loop, but appears sometimes to be ring-shaped (Text-fig. I, 17). When swollen it takes the stain deeply and presents a striking appearance as it lies in a halo of deep blue-staining cytoplasm. Then a septum is formed separating the spore part of the cell from the remaining part of the mother cell (Text-fig. I, 17, 18). At this stage the three remaining 'chromosomes' stain only faintly and are often very irregular in appearance; they may be connected with each other. Pl. 2, phot. 7, shows a row of five spore mother cells in which the spore 'chromosomes' are not yet distinguishable. The two cells on the left-hand side of the photograph show their structures more clearly than the others. Above these two cells a spore mother cell is seen with a well-differentiated spore part, the big-spore 'chromosome' lying in a uniformly stained portion of the

cytoplasm; the rest of the original cell is stained more faintly and contains three nuclear structures connected by chromatin strands. The five spore mother cells illustrated in Pl. 2, phot. 5, are in about the same stage of development. Their stout-spore 'chromosomes' were loop-shaped in the actual preparation. The remaining 'chromosomes', more or less connected with each other, are disintegrating.

FUSION OF NUCLEAR STRUCTURE INDUCED BY AN EXTRINSIC STIMULUS

In all the spore-forming organisms so far examined fusion of nuclear material occurs during development. This process never takes place in the early stages of growth, but may occur after 12–24 hr. incubation, gradually involving more and more cells. The time of its occurrence varies with the species, the individual strain, the amount and quality of the inoculum, the conditions of the medium and other factors.

Fusion of the 'chromosomes' can be induced suddenly in all the anaerobic spore-bearing organisms that have been tested. To demonstrate the induced fusion, surface-plate culture of *Cl. welchii*, *Cl. septicum* and *Cl. oedematiens* var. *gigas* were incubated anaerobically for various short periods. Impressions fixed as quickly as possible after opening the jar showed cells with 'chromosome' patterns as illustrated in Text-fig. I, 1, and Pl. 2, phot. 1, as well as in Pl. 3, phots. 9 and 10. Preparations fixed after 7, 15, 30 and 60 min. exposure to air showed varying degrees of fusion of the nuclear apparatus of the cells according to the particular organism tested and the time of exposure. Thus, of the three organisms mentioned above, *Cl. oedematiens* var. *gigas* is the most sensitive and *Cl. welchii* the least sensitive to air exposure. The different degrees of sensitivity are reflected in the time intervals elapsing until the induced nuclear fusion is completed. *Cl. oedematiens* var. *gigas* presented a marked transformation of its cells after only 7 min. exposure to air. Phot. 10 demonstrates the configuration of the chromatin immediately after opening the jar. In contrast to this, Pl. 2, phot. 11 shows the changes after 7 min. air exposure. The axial chromatin rod has already formed in many cells (*c*); in others the chromatinic strands are still in the process of fusion and an irregular outline of the rod can be detected (*b*); in one particular long cell many short 'chromosomes' are seen to be scattered in the longitudinal axis of the cell (*a*). The cytoplasm of the fusion cells has become transparent, and the membranes and transverse septa show very clearly. After 15 min. exposure to air all the nuclear structures have fused (Pl. 3, phot. 8). When the culture plate that had been used for the 7 and 15 min. test was left exposed to air for 24 hr. the chromatinic material had become concentrated into a darkly stained round ball in the middle of each cell (Pl. 3, phot. 12). Although this last stage has not been observed in *Cl. septicum* and *welchii* the fusion of the dumbbell bodies takes place much in the same way as in *Cl. oedematiens* var. *gigas*, though more slowly. *Cl. welchii* showed a marked change of chromatin structures after half an hour's exposure to air (Pl. 2, phot. 6). After an hour's exposure the transformation was complete (Pl. 2, phot. 2). Although a strong similarity between the natural and the induced fusion exists, the induced fusion cells neither produce spores under aerobic conditions nor do they continue the process of sporulation when anaerobic conditions are restored. Often growth completely ceases after air exposure, and this is particularly true of *Cl. oedematiens* var. *gigas*; or if growth continues, it seems to do so reluctantly.

Induced fusion does not occur during the very first hours of development, but it takes place already at a time when in parallel cultures, not exposed to air, the natural fusion has not yet started. Fusion provoked by air exposure quickly involves all the cells of the culture; it occurs more rapidly where the growth is thin and more slowly where it is thick.

FUSION OF NUCLEAR STRUCTURES IN *SPHAEROTILUS NATANS*

This micro-organism lends itself exceptionally well to studies of nuclear transformation owing to the width of its elements. Its young forms are motile; at a later stage of growth long, immobile, sheathed filaments appear. I became interested in this organism on account of a special staining property it possesses, and because a fusion of nuclear material occurs at a certain stage of cultivation. When I tried the method for demonstrating bacterial chromatin on *Sph. natans* I found that it was of no advantage to apply hydrochloric acid treatment, and that its nuclear apparatus showed brilliantly if, after osmic acid fixation, the cells were stained with Giemsa solution. The same is true for the *L* 1 organism as described previously by myself (1942). This experience with *Sph. natans* proves that the demonstration of the chromatinic dumbbell bodies is not necessarily dependent on the hydrochloric acid treatment.

The young motile cells, which are short and oval, possess either one stout chromatin body or two in a parallel, crosswise or V arrangement, indicating that they have developed out of one structure by longitudinal fission. In addition longer cells are found which contain two pairs of dumbbell bodies. All these elements are division stages either on the way towards growing into a two-cell or a four-cell

filament. When long threads have formed, chromatin structures similar to those in the motile cells can be seen inside the elements composing the sheathed chains of bacteria (Text-fig. I, 19, and Pl. 4, phot. 13 *a*). As the filaments grow older more 'chromosomes' appear in the individual, now elongated, organisms. At this stage the cells are multinucleated and contain a dense, centrally placed packet of chromatin structures (Text-fig. I, 20 and Pl. 4, phot. 13 *b*). Each of these darkly staining bodies shown in the illustrations represents a number of dumbbells which have drawn together so closely that the single elements can no longer be detected or have fused together. In the latter event *Sph. natans* would furnish another variety of nuclear fusion, since the fusion body is not an axial cylinder, but fills the whole centre of the cell and is formed by a drawing together of the dumbbell bodies.

DISCUSSION

As early as 1901 Nakanishi studied spore formation in *Clostridium tetani* by means of a special staining technique. He then observed what he termed the 'nucleus' which, as he pointed out, may consist of two rod-like structures and is usually present in that part of the spore mother cell which does not contain the spore. In addition he found, rarely, another 'nucleus' in the other part of the spore mother cell. These observations indicate that he must at times have seen three nuclear bodies inside the spore mother cell apart from the spore, corresponding to the three extruded elements here described. The significance of this correct observation was apparently not understood by Nakanishi. In 1909 Dobell studied spore formation in preparations fixed with osmic acid and stained with Giemsa. His observations comprise a number of interesting but isolated findings. In the light of new knowledge they can be linked up together, and various cell appearances he describes fit well into our scheme of the cycle passed through during sporulation. He interpreted correctly the double-spore mother cell that previously had been regarded as a sexual stage by Bütschli and that has been described here for *Cl. oedematiens* var. *gigas*. Pietschmann & Rippel (1932), when studying spore formation by means of the Feulgen reaction, likewise noted nuclear structures outside the spore. Their correct description of the situation of these chromatin elements inside the remaining part of the mother cell ('Mutterzellrest') is not accompanied by any commentary which attempts, as in the present study, to explain the facts by reference to the preceding development of the cell.

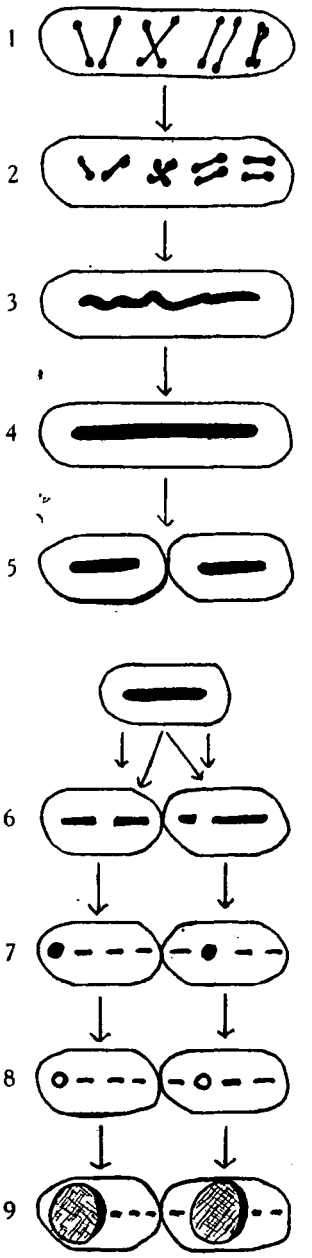
Badian (1933) is the first author to describe a process of 'autogamy' followed by a reduction partition in spore formation of bacteria. He studied *Bacillus subtilis* and *B. mycoides* and found that both formed their spores in the same way. He used osmic acid vapour for fixation and stained with Giemsa solution; the chromatinic structures became visible after a simple differentiation with eosine. Although his photographic evidence is somewhat defective and his drawings are merely diagrammatic, his description of the process of spore formation is very clear. His main observations have been confirmed in the present paper and may be summarized thus: spore formation is initiated by a process of fusion of the chromatinic dumbbell bodies; this is followed by a splitting of the fusion body into four 'chromosomes'; and from these one is included within the spore and three perish. Although my results agree with Badian in these important main features of the development, there are a number of points of disagreement. According to my observations: (1) fusion takes place in cells that contain more than two chromosomes; (2) the longitudinally situated fusion cylinder does not regularly turn into another position before division takes place; (3) when it does split up, this process occurs not in one but in two steps as described here, and I cannot confirm the fan-like partition as drawn by Badian; (4) the spore 'chromosome' has usually a loop- or ring-like shape (sphere?) and is not a mere dumbbell body as shown in Badian's diagrams; (5) Badian has not observed the membrane which divides the young developing spore from the spore mother cell; and (6) he has not been able to demonstrate the nuclear structure in the mature spore. The main difference between Badian's description and the one here presented is that he seems to have compressed his observations into a very rigid scheme that is not always in conformity with the natural development, whereas I have tried to illustrate, in highly magnified photographs and in drawings which follow closely the microscopical pictures, the great variety of configurations of the nuclear structures during sporulation. And yet I have been able to show that the development follows the same main principles in different bacterial species. In spite of the criticism here I should like to point out that it is greatly to Badian's credit to have been the first to discover the main features of chromatin fusion with subsequent reduction of the chromatinic matter at a time when the Feulgen reaction had hardly been applied to bacterial organisms. As early as 1930 Badian had studied the nuclear apparatus of a *Myxobacterium*, and had given descriptions of the cytological development of a second *Myxobacterium* in 1933 and of Actinomycetaceae in 1936. From these studies it would seem that the so-called spores in bacteria and in the two other great groups of micro-organisms may not be comparable elements from the cytological point of view. Reinvestigation of these micro-organisms might yield valuable in-

formation on their taxonomic position. Stille (1937) reported that one part of the chromatin substance is included in the spore and that the second part of the chromatin substance disintegrates with the spore mother cell. He, like Piekarski (1940), has, however, not observed the fusion process, neither has he noted that finally four (not two) nuclear structures are present in each spore mother cell. In 1939 Allen, Appleby & Wolf published an investigation of cytological appearances in a spore-forming bacillus. Although they describe the occurrence of 'haploid' and 'diploid' cells, this statement seems to be more an assumption than one based on direct observation of the fusion process that leads to what is called 'diploid' organisms. These authors have also observed a reduction partition in certain spore mother cells, and have concluded that in one kind of spore mother cell one of the resultant 'chromosomes' forms the spore while the others seem to be extruded. Their main assertion that different modes of spore formation occur in one and the same organism is not in agreement with my observations, which have shown that sporulation in four different species—one aerobic and three anaerobic—follows the same principles. In 1941 Beebe described the cytology of *Myxococcus Xanthus* n.sp. His account of the development of its spores differs from Badian's and from the description here presented, for he observed that the fusion cell forms the spore directly, and that the reduction of the chromatinic material seemingly takes place during spore germination. Beebe's evidence consists of photographs and drawings which are not very convincing.

Discrepancies of this kind are not surprising, for it is not easy to follow the sequence of events, since various stages of development are often present side by side in one preparation. An exceptionally good technique for demonstration and extensive material for observation are essential if the findings are to be interpreted beyond reasonable doubt. It is desirable to re-examine sporulation in Myxobacteriaceae and Actinomycetaceae by the methods here employed and to compare the findings with those revealed in the present study for spore-bearing bacteria. Finally, it should be emphasized that, though the process of nuclear fusion and the reduction partition here described resemble to a certain extent nuclear transformations in higher organisms, they are probably more primitive and simpler than these. Expressions such as 'autogamy' and, in particular, 'meiosis' should not be used unless with mental reservation; the same caution applies to the expression 'chromosome'.

SUMMARY

Changes of nuclear structure in bacteria have been studied by means of the hydrochloric acid-Giemsa method which produces brilliantly stained specimens and can be carried out with almost the same ease as some of the ordinary routine staining techniques.



Text-fig. III. Outlining the nuclear changes in the four spore-bearing organisms studied in this paper.

The nuclear changes in the four spore-bearing organisms studied are outlined in Text-fig. III, to which the following numbers refer. The dumbbell bodies which are dispersed in the cells of the young growth (1) become alined in the long axis of the

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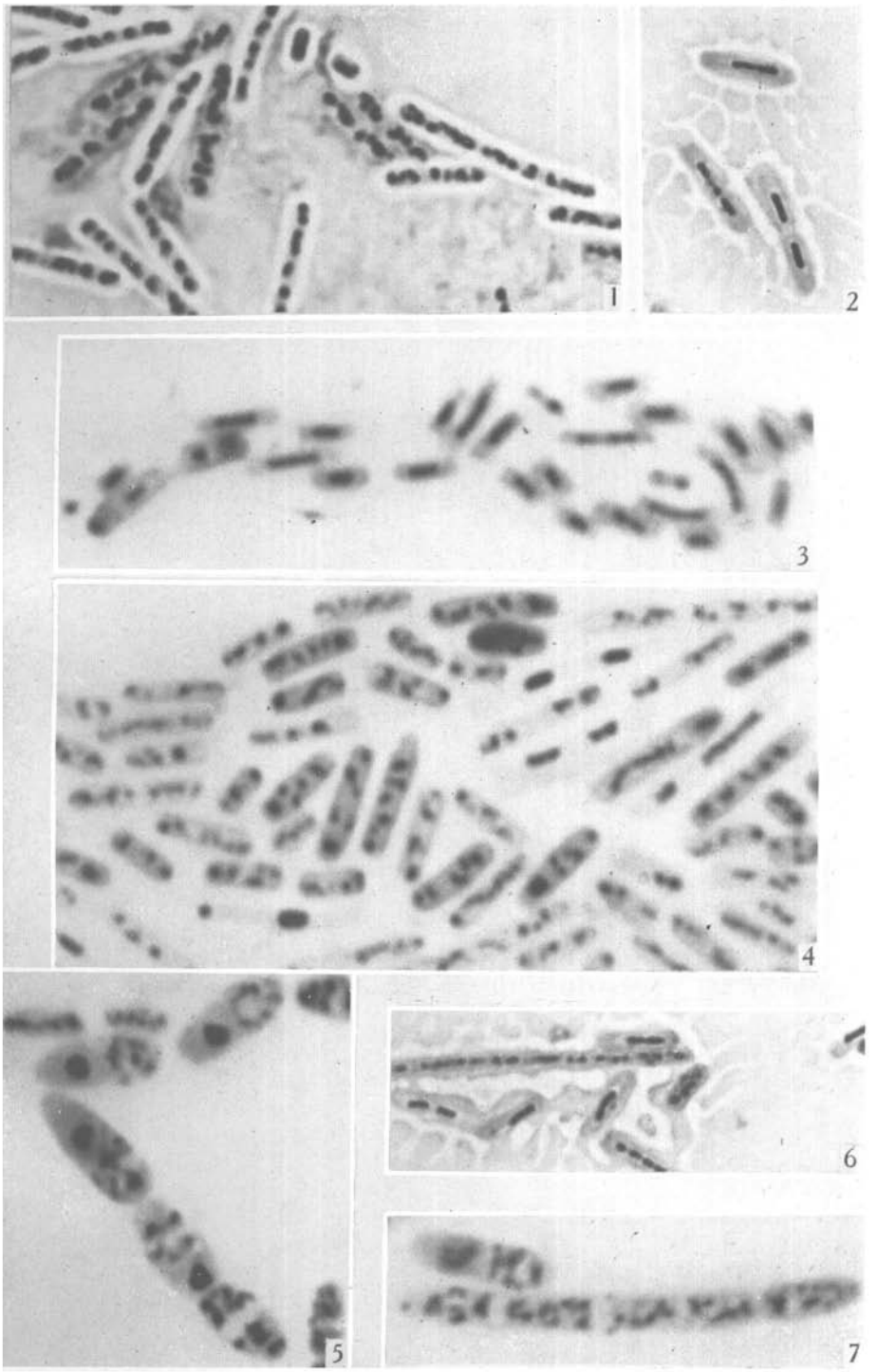

Phots. 1–7.

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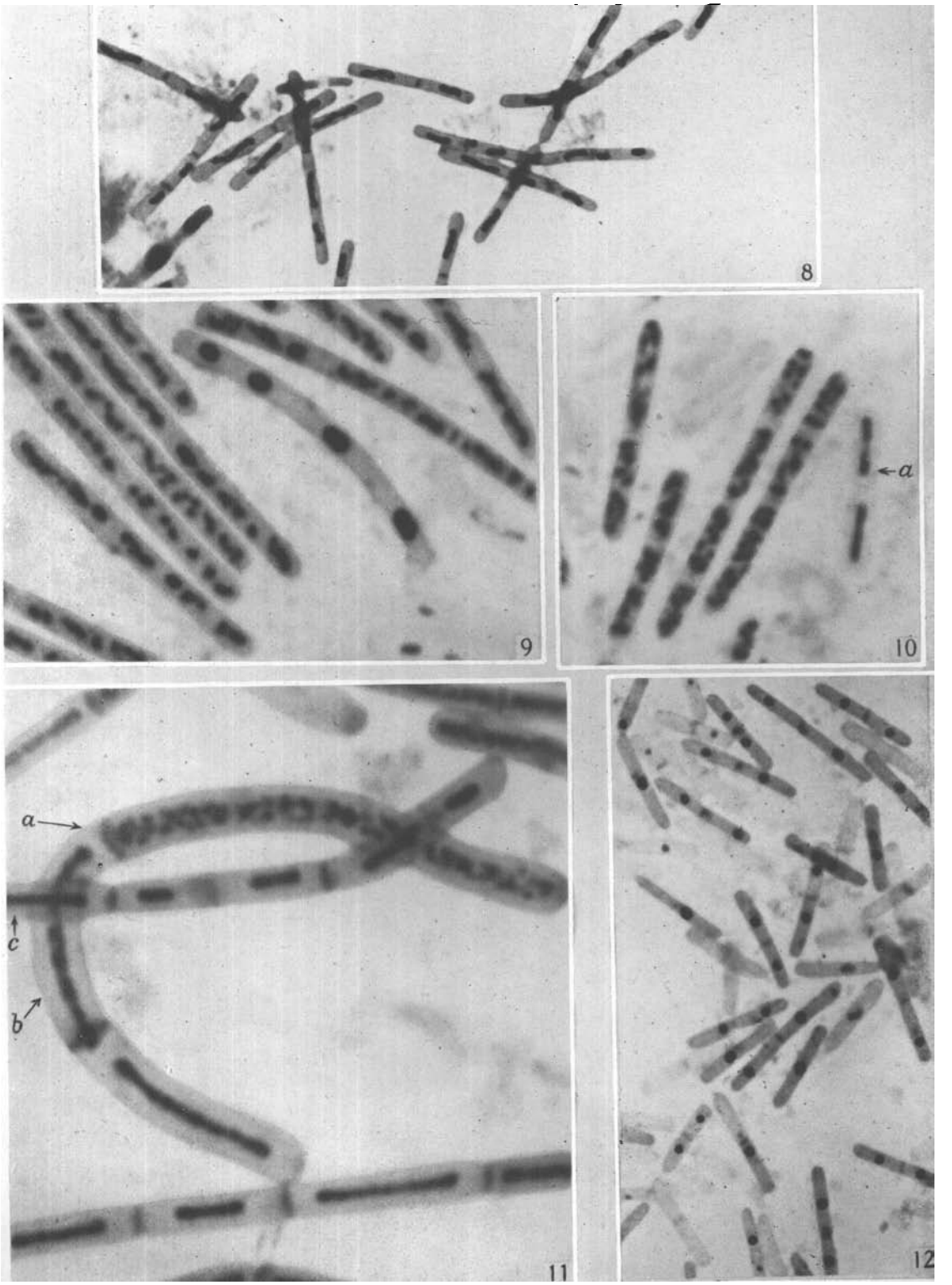

Phots. 8–12.

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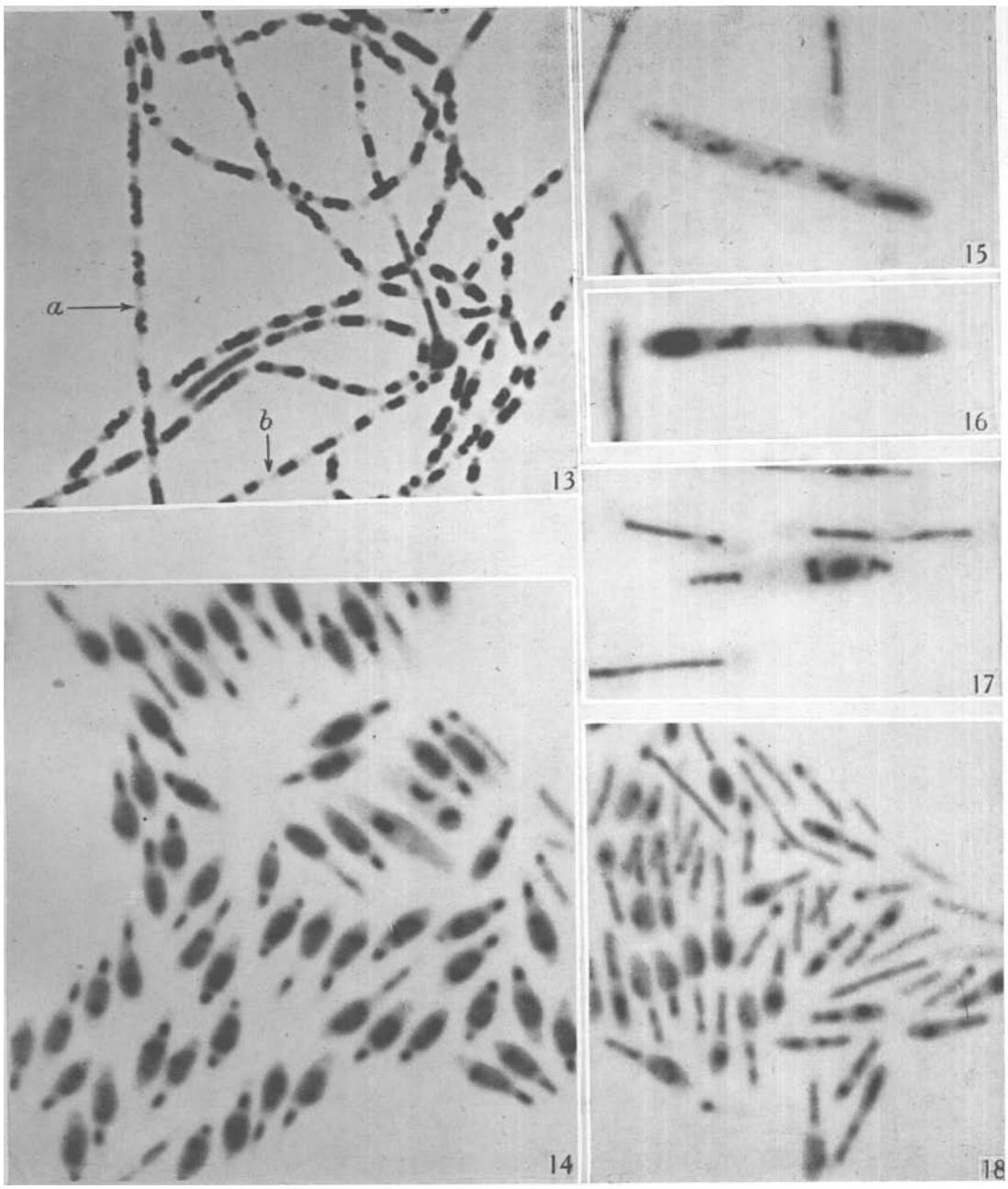

Phots. 13–18.

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cell (2) where they eventually fuse into an axial nuclear cylinder (3, 4). These cells divide up into fusion cells of approximately the same length (5). The development of the 'chromosome' stage (1) into the fusion cell (5) is the first step in the process of sporulation. During its further development the fusion cell or spore mother cell divides twice (6, 7), with the result that it is segregated into four structures which often assume dumbbell shape. Therefore the chromatin cylinder of the individual spore mother cell seems to be equivalent to four nuclear elements one of which functions as the spore 'chromosome' ('nucleus'?), whereas the remaining three disintegrate (8, 9). The ripe spore (9) representing, as it does, the smallest cell unit contains one nuclear structure only.

Therefore the two main features in spore formation of bacteria appear to be (1) a fusion of the dumbbell bodies into an axial chromatin rod ('autogamy'?), (2) a reduction partition which is reminiscent of, though not corresponding to, the more complicated phenomenon of meiosis in the higher organisms. The sporulation, as outlined in this paper, gives new proof of the important part played by the chromatinic dumbbell bodies ('chromosomes') in the developmental cycle of spore-bearing organisms. The fusion cell with its axial chromatin cylinder has for the first time been proved to have a progressive functional significance as a stage in a nuclear cycle. The particular mode of fusion followed by reduction partition suggests that the chromatinic dumbbell bodies may be concerned with the transmission of the hereditary characters in bacteria.

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EXPLANATION OF PLATES 2–4

PLATE 2

Phot. 1. *Clostridium welchii*, young, 5 hr. growth, magn. × 2800.

Phot. 2. *Clostridium welchii*, fusion of nuclear structures after 1 hr. exposure to air; magn. × 1875.

Phot. 3. *Clostridium welchii*, growth about 15 hr. old; 'fusion cells'; magn. × 3500.

Phot. 4. *Clostridium welchii*, 1–2 days' growth; various cells have completed their second nuclear division; magn. × 3500.

Phot. 5. *Bacillus mycoides*, spore mother cells with spore part clearly developed and extruded, chromatinic material decaying; magn. × 3500.

Phot. 6. *Clostridium welchii*, fusion of nuclear structures after ½ hr. exposure to air; magn. × 1875.

Phot. 7. *Bacillus mycoides*, spore mother cells after completion of nuclear divisions, spore chromosome not yet differentiated; magn. × 3500.

PLATE 3

Phot. 8. *Clostridium oedematiens* var. *gigas*, fusion of nuclear structures has occurred after 15 min. exposure to air; magn. × 1875.

Phot. 9. *Clostridium oedematiens* var. *gigas*, natural fusion of nuclear structures has started in some cells. In the middle a double spore mother cell is seen which has already completed the first division step of the nuclear cylinder; magn. × 3500.

Phot. 10. *Clostridium oedematiens* var. *gigas*, young, about 8–12 hr. growth; dumbbell bodies are seen lying across the cells; magn. × 3500.

Phot. 11. *Clostridium oedematiens* var. *gigas*, various degrees of fusion after 7 min. exposure to air; magn. × 3500.

Phot. 12. *Clostridium oedematiens* var. *gigas*, nuclear fusion after 24 hr. exposure to air; magn. × 1875.

PLATE 4

Phot. 13. *Sphaerotilus natans*, edge of a culture grown on a coverslip in liquid medium showing sheathed filaments containing young cells with chromosomes in V arrangement in (*a*) and cells with fusion bodies in (*b*); magn. × 1875.

Phot. 14. *Clostridium septicum*, mature spores with chromatinic tags on one or on both sides; magn. × 3500.

Phot. 15. *Clostridium oedematiens* var. *gigas*, double spore mother cell forming a spore on either end of the filament; six nuclear structures can be detected between the two developing spores; see Text-fig. II, 21, 22, 23 corresponding to phots. 15–17; magn. × 3500.

Phot. 16. *Clostridium oedematiens* var. *gigas*, cell of the same type as illustrated in phot. 15; magn. × 3500.

Phot. 17. *Clostridium oedematiens* var. *gigas*, single spore mother cell with developing spore and three extrasporal chromatinic bodies; magn. × 3500.

Phot. 18. *Clostridium septicum*, young spores are developing; occasionally two or three round or rod-like nuclear bodies are to be seen in the mother cell apart from the spore; magn. × 3500.

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