THE CHROMATIN BODIES OF BACTERIA

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It has now been established in several different ways that the protoplasm of bacteria is differentiated into a ground cytoplasm and one or more relatively large structures that differ from the cytoplasm in texture and in giving a positive Feulgen reaction. The *existence* of these entities, whose behavior towards stains has earned them such names as "nucleoids" or "chromatin bodies," is not in doubt, but uncertainty persists about their relationship to the nuclei and chromosomes of other protists and of higher organisms. It is the purpose of this article to provide

some viewpoints for a discussion of this relationship.

I. OUTLINE OF VARIETIES OF NUCLEAR BEHAVIOR IN HIGHER ORGANISMS AND PROTISTS

A nucleus has probably been looked for in bacteria ever since Koch recommended the use of aniline dyes to bacteriologists 80 years ago. A review by Delaporte (1), published in 1939, already listed over 400 papers on the subject. At that time few bacteriologists were interested in the anatomy of bacteria. Now many are and the literature on bacterial cytology is extensive.

Although much work has been done it seems to have been lacking in something, for after discussing the bacterial nucleus for over 70 years bacteriologists are still far from agreeing about its structure and behavior. This is odd in a subject based on observations on relatively simple cells and has no parallel in other branches of morphology. The causes of this situation are many, and few of them are technical. An important one is lack of familiarity with current cytological thinking. Bacteriologists, for example, are still prone to discuss the problem of the bacterial nucleus under a number of headings such as: "the naked nucleus," "the diffuse nucleus," "the chromidial nucleus," or "the true, vesicular nucleus." This list of alternatives, which was last used quite recently, is usually ascribed to Lewis (2). But he was not its main author. Five of Lewis' eight points were already debated by Douglas and Distaso (3) in a paper published in 1912.

Terminology of 40 years ago no longer adequately describes the increased information and deeper understanding which cytologists have since acquired. The continued use of this terminology by bacteriologists of today is scarcely defensible.

Of the numerous observations on bacterial nuclei which have accumulated over the years many, though separated by decades, are in good agreement. Interpretations, not facts, have remained controversial. Recurrent disagreements over interpretations reflect the absence of agreed standards of truth and validity and of a common terminology. Workers in the various fields of general cytology are not handicapped by either of these shortcomings, and it is from the point of view of this discipline that the chromatin structures of bacteria will be discussed in the present article. This enterprise requires some acquaintance with cytological terminology. To provide it the description of bacterial chromatin structures will be preceded by a brief and simplified account of the main varieties of nuclear structure and behavior now recognized by cytologists. Throughout the discussion "higher plants and animals" will frequently be contrasted with "protists" and it seems advisable to define the meanings of these designations at the outset. Following the arrangement proposed by the authors of the *Review of medical microbiology* (4) (in collaboration with Dr. R. Y. Stanier) the "protists" are taken to include the protozoa, slime molds, fungi, the various divisions of the algae, the blue-green algae and the bacteria. "Higher organisms" are the plants and animals above this level. This classification emphasizes important differences in the organization of individuals. In the higher organisms the mature individual is, in most instances, composed of many classes of variously specialized cells. This mass of cells is integrated into a unit of activity with the aid of systems of nerves and/or circulating humors.

Protists may be either unicellular or many-celled organisms. In protists with many cells, *e.g.*, higher fungi or algae, vegetative growth is unrestricted and an individual not easily defined. Most of the cells of a given organism are alike, few are differentiated for the performance of special tasks such as the formation of vegetative spores, sexual reproduction, the trapping of nematodes or rotifers, and there are no tissues of specialized cells to integrate the cell mass into an individual.

To separate the higher organisms from the protists may not be meaningful to geneticists or biochemists, but it is useful in the present context because it goes hand in hand with an important difference in nuclear behavior in the two groups. In the higher organisms the course of nuclear division has become stereotyped, whereas the protists have retained so many strikingly different ways of segregating sets of chromosomes that there is not a single area of this kingdom without its unsolved nuclear problems (5, 6).

A. Definitions

A definition of the cell nucleus which has proved valuable because it has stimulated research is that given by Belar (7, p. 506) and Geitler (5), which regards as nuclei only those

structures which are a collection of *chromosomes*. Nuclei, thus defined, arise from chromosomes and later may or may not give rise to chromosomes again. Chromosomes, which this terminology regards as the essential and irreducible element of nuclei, are complex both chemically and morphologically and, like first violinists, more easily recognized by their characteristic *behavior* in a specific situation (nuclear division, concert performance) than outside it. All the properties ascribed to chromosomes are not equally well displayed in all and every species. Among their most important features are individuality, differentiation along their long axis, their occurrence in sets of nearly constant numbers, a cycle of spiralization and relaxation during nuclear division and, lastly, their mode of multiplication. Where the chromosomes are large enough to be resolved in the microscope, it has invariably been found that they divide longitudinally. Elements that divide transversely are not chromosomes.

The chromosome of the present discussion is the chromosome of morphology. It is something that can be seen and in favorable material can be handled under the microscope. It is an object which has a certain, well-defined anatomy. It is not necessarily the chromosome of genetics, which is a synonym for "linkage group" and may or may not have a visible equivalent.

Belar's definition is strictly morphological and takes no account of the fact (not precisely known in his time) that chromosomes almost invariably contain compounds of deoxyribonucleic acid (DNA). This association is so common that the presence in them of DNA must now be regarded as a prominent feature of nuclei. If we overemphasize this point we shall find ourselves in an awkward position regarding the blue-green algae and other protists, to be described later, whose cells possess organelles which divide in a regular manner, are charged with DNA but lack visible chromosomes. By morphological standards such structures are not comparable to nuclei and it will be best to call them by a distinctive name such as "chromatin bodies."

B. Nuclei

1. The Phase of Rest

The appearance of the resting nucleus reflects the condition of the chromosomes within. In many types of cells of the higher organisms the chromosomes appear to be maximally uncoiled and hydrated and fill the nuclear vesicle completely. In this condition they are poorly stainable except for specialized "heterochromatic" segments in which the concentration of DNA remains high. This is not the only type of resting nucleus to be found in higher organisms, but it is the most common one.

In protists there is greater variety of nuclear design. Of special interest are those resting nuclei of protozoa in which the chromosomes remain more nearly in the condition of the division phase than they commonly do in higher organisms. Nuclei of this kind, *e.g.*, those of euglenoids and dinoflagellates and the macronuclei of *Euciliatia* and *Suctorea* are very chromatinic, give an intensely positive Feulgen reaction and in life appear packed with a multitude of small bright granules (8, 9). Too detailed a description of known varieties of nuclear morphology diverts attention from the basic fact that all nuclei have in common—that they arise from chromosomes. It follows that different types of nuclei are most usefully classified not on the basis of the morphology of the resting stage, which may vary from tissue to tissue in the same organism, but according to the behavior of the chromosomes during division.

2. Division

The multiplication of sets of chromosomes is accomplished in different ways by different organisms. Three types are well recognized, and a fourth one at present appears as an attractive possibility.

Mitosis. Mitosis is the common mode of division of the nuclei of higher plants and animals; it is also seen, with many modifications of detail, in the protozoa, algae and during the formation of sexual spores of fungi. Its essential feature is the duplication and orderly segregation of the whole set of chromosome individuals of a given nucleus. In the most widely known form of mitotic division the chromosomes coil themselves into a much more compact shape than they have during the resting stage, become deeply stainable, align themselves on a fibrous spindle (which may or may not be anchored in centrioles) and split lengthwise. The split halves are then pulled, or actively glide, to opposite poles of the cell where new chromosome chambers or nuclei are constructed around them.

In the best known forms of mitosis the ana-

phase movements of the chromosomes are mediated by a spindle apparatus, but it has long been known that divided chromosomes may also segregate without the aid of a spindle. This and other varieties of the behavior of chromosomes during division, such as their failure to spiralize (*e.g.*, in *Coccidia*) have led to the recognition of many different types of mitosis. Two features are common to them all: the emergence of separate, recognizable chromosomes and a clear-cut difference between the appearance of a nucleus in mitosis, with its chromosomes on the spindle or aligned by other means; and a nucleus at rest, with its chromosomes visibly or, more frequently, invisibly clustering together in a compact and random arrangement. In other words, the movements of the chromosomes may be difficult to understand, their numbers uncountable, and neither centrioles nor spindle discernible, but there is never a doubt whether one is looking at a resting or a dividing nucleus! This wants emphasizing because other modes of dividing a nucleus do occur in which the transition of stages of rest and stages of division is continuous.

Endomitosis. This is the form used to describe the division of randomly arranged chromosomes inside the nucleus without the aid of a spindle apparatus. It is usually not followed by division of the nucleus itself. Repetitions of this process may lead to high degrees of polyploidy. Endomitosis occurs in higher plants and animals and in protozoa. It is probably in this way that the macronuclei of the *Euciliatia* attain their prodigious size (10, 11). Our knowledge of endomitosis is largely due to the work of Geitler (12).

Amitosis. This is the direct division of a nucleus without the emergence of chromosomes and without a spindle apparatus. It is the normal mode of division of the macronuclei of ciliated protozoa. These organisms have two kinds of nuclei—a relatively large macronucleus and a much smaller micronucleus; and there are two modes of reproduction—vegetative multiplication, which is simple fission, and fission preceded by conjugation (or, in some instances, autogamy). Both nuclei divide in the course of vegetative multiplication, the macronucleus directly, the micronucleus by a process superficially resembling mitosis. In a dividing micronucleus filamentous chromatinic bodies segregate to the poles of a barrel-shaped spindle structure. What it is that is being divided here has been rendered doubtful by the demonstration that the chromatinic filaments in the micronuclei of many ciliates are connected laterally, divide transversely, and cannot therefore be regarded as chromosomes (13). That chromosomes must somehow be represented in these filaments follows from the observation that typical chromosomes are formed in micronuclei during the meiotic divisions that take place after conjugation. The macronucleus does not take part in conjugation and disintegrates during this process. A new one is formed through repeated endomitotic divisions of one of the postconjugal micronuclei. It is thus certain that the macronucleus arises from chromosomes even though they are not individually discernible either during or between divisions in the fully grown organ.

It is difficult to imagine how duplicates of all the individuals of a set of chromosomes can be equitably distributed between the halves of a directly dividing macronucleus. That it is somehow done with considerable, if not absolute, efficiency is evident from the researches of Sonneborn (14, 15) [reviewed by Beale (16)] who showed that in *Paramecium aurelia* it is the genes in the *macronucleus* which determine the animal's performance and phenotype, and that the micronucleus is dispensable and when present has little effect on the life of the animal. Equally surprising has been the discovery [summarized by Corliss (17)] that there are strains of the ciliate *Tetrahymena* which have either lost or never had a micronucleus. Such strains, also known in *Paramecium*, have already been maintained in laboratories for over 10 years and have also several times been encountered in natural environments. Apparently the maintenance of these highly differentiated animals is satisfactorily mediated by a nucleus which plainly divides only by amitosis.

Direct division of nuclei is not restricted to ciliate protozoa. The older mycologists held that vegetative nuclei of certain fungi divide directly (18, 19). Although this belief has now fallen into disfavor, during the past few years the writer and his collaborators have obtained much fresh evidence that the segregation of the chromosomes in dividing vegetative nuclei of species of *Saprolegnia*, *Mucor*, *Phycomyces*, *Penicillium*, and *Geotrichum* is indeed carried out in a singularly direct manner involving neither spindles nor metaphase plates (unpublished data). There is also much

that is odd and unsuspected about the structure of *resting* vegetative nuclei of these fungi. These matters are likely to remain controversial for some time to come, but their mention at this point will perhaps serve as a warning that it would be unwise to lay down in advance what mode of division any particular class of protists may be expected to practice.

Genome segregation. This is a new concept introduced by Grell (20) as a tentative explanation of the strange behavior of the chromosomes in certain forms of nuclear division found in protozoa (*Peridinidae*, *Radiolaridae*, *Euglenida*). There the chromosomes, usually very numerous, appear completely divided at prophase. No spindle is formed, and the mass of randomly distributed twin chromatids is segregated into two lots by the gradual constriction of the nucleus. The oddest feature of this mode of division (essentially an endomitosis immediately followed by division of the nucleus) is the fact that sister chromatids move to the *same* pole. Obviously all the chromosomes must here be homologous. A nucleus filled with many duplicates of one single chromosome rather than with sets of different ones seems strange, and Grell has found evidence that, at least in one protozoan, the radiolarian *Aulacantha scolymantha*, the alleged "chromosomes" are not what they seem.

At certain stages of the division process, the chromosomes of this organism, estimated to number more than a thousand, are long smooth filaments, but at other times they appear as chains of segments of different length and shape. The sequence of segments is the same in different "chromosomes," and Grell has suggested that the segments themselves are the real chromosomes and that each chain represents one haploid set of them. Instead of having more than a thousand "chromosomes" *Aulacantha*, according to this view, has as many duplicates of its whole haploid set of chromosomes, or "genomes."

These observations are of great general interest because they provide the glimmering of an explanation of the at present incomprehensible behavior of macronuclei, especially of those which divide not by constriction into two equal portions but by single successive or multiple, simultaneously arising buds. If macronuclei could be regarded as vast agglomerations of genome duplicates, then it would no longer be astounding that even fragments of them (*i.e.*, small numbers of genomes) are genetically adequate.

C. Chromatin Bodies (Nonchromosomal Nuclei)

There are several kinds of protozoa which possess chromatin organelles that do not arise from chromosomes and do not give rise to them during division. Such structures, which by the standards of Belar and Geitler are not nuclei, are found in intracellular and blood parasites of the subphylum *Sporozoa*, *e.g.*, in *Theileria*, *Babesia*, and *Nosema*. Nuclear behavior in these organisms is, admittedly, incompletely explored but it does not now seem likely that it will become amenable to a description in terms of (morphological) chromosomes. A nonchromosomal nucleus has been described with great clarity by Chen (21) in an avian malaria parasite, *Plasmodium elongatum*. Figure 1 shows that the central, Feulgen positive "nucleus" of the schizonts is at all times of the same degree of Feulgen positiveness. Phases of "rest" merge almost imperceptibly into phases of division, and although dividing nuclei are distinguished by hoops (circlets, bands) of chromatin, the behavior of these bands in no way resembles that of chromosomes.[1] If Belar (7) and

[1] Chen's account of nuclear behavior in *P. elongatum* is exceptional only in the clarity of its illustrations. The mode of division of the schizont nuclei of other plasmodia appears at present equally obscure. In the course of a detailed description of the development of *P. gallinaceum* Huff and Coulston (22) emphasize that: "... although many hundreds of schizonts have been studied in all stages of growth no evidence has been seen of mitotic behavior." Moreover, Chen's findings as well as those of Huff and Coulston are corroborated by the results of a careful study, on modern cytochemical lines, of *P. gailinaceum*, *P. lophurae*, and *P. vivax*, by Lewert (22*a*) who has this to say on schizont nuclei: "The morphology is relatively uniform except for those stages interpreted as being division forms. Immediately prior to the last division the nuclear material is elongated and in some instances seems to be separated into two connected, roughly bar-shaped masses. ... In no instance was there any evidence of the concentration of Feulgen-positive material in discrete particles or granules." It does not follow that the behavior of the nuclei is equally inscrutable at all stages of the life cycle of plasmodia. Orderly chromatin arrangements have been seen in gametocyte nuclei of *P. falciparum* by Luedicke and Piekarski (22*b*) which have been interpreted as stages in a process of genome segregation. But this suggestion is still only tentative.

The strangeness of the behavior of *Plasmodium*

Geitler (5) do not regard structures with such properties as nuclei, they are not excessively pedantic. If chromosomes are discarded as the essential property of nuclei and if we widen the term "nucleus" to include all and every Feulgen positive, organized, visible structure "endowed with genetic continuity," then there is no reason why the weird kinetoplast of trypanosomes, *Bodo* and other zooflagellates should not also be regarded as a nucleus. The term "kinetoplast" has been applied to a variously shaped body which in the aforementioned species lies close to the blepharoplast (at the base of the flagellum). Kinetoplasts are Feulgen positive and divide directly; their function is unknown. Trypanosomes may lose their kinetoplasts permanently, either spontaneously (23) or through cultivation in the presence of pyronin or acridin, without ill effects on the life of the cell or its descendants.

The protozoa whose nuclei have just been described are all very small, and it may reasonably be argued that this is the cause of the uncertainty regarding the behavior of their nuclear structures. This cannot be said of the best known example of a nonchromosomal nucleus, namely, the voluminous network of chromatinic, Feulgen positive (1, 24) threads and granules which occupies the center of the cells of the blue-green algae. The central chromatinic body or "chromidial apparatus" of these organisms, as is now well known, divides by gradual constriction without undergoing visible, intelligible changes of its fine structure. Obviously, as Geitler (5) has emphasized: "It is improbable that the division of the central body . . . is really as disorderly as it *appears* to be. We must assume that smaller units are concealed in the chromidial apparatus and that these show regularity of mass and number." For a detailed, magnificently illustrated, modern account of the central bodies of blue-green algae and a full discussion of the older literature, see Cassel and Hutchinson (25).

A discussion of the *Cyanophyceae* is very pertinent to our inquiry because they are more closely related to the bacteria—despite important differences between them—than to any other group of organisms. The same range of basic shapes is found in both groups. Were they to lose their distinctive pigments those blue-green algae which have the shape of cocci, rod forms, or helical cells would be hard to distinguish from their counterparts among the bacteria. By light microscopical standards the structure of the cells of blue-green algae and bacteria is simple. Bacteria have means for the imparting and exchange of genetical information which have so far not been encountered in the *Cyanophyceae*, but it is true of both groups that they form neither gametes nor other visible devices for sexual reproduction. Close relationship may also be inferred by the presence of diaminopimelic acid which has so far been encountered only in blue-green algae and bacteria (26).

D. Summary

1. There are two kinds of nuclear structures, those constructed from chromosomes and those not demonstrably so constructed.

2. The nuclei of cells of higher organisms are constructed from chromosomes and divide by processes of mitosis which follow essentially the same course everywhere. In some tissues of certain higher plants and animals the nuclei grow by endomitosis. Macronuclei of protozoa also seem to arise by this process.

3. Chromosome-nuclei of protists divide by mitosis or in one of several other ways (amitosis, genome segregation) not known (or unimportant) in higher organisms.

4. In the chromatin bodies of some protists chromosomes cannot be demonstrated. The best known example, but not the only one, is the "chromidial" central body of the cells of the blue-green algae, a group of organisms which has much in common with the bacteria.

5. There is monotony of nuclear behavior in higher organisms, but there is much variety of nuclear structure and behavior in protists.

nuclei is emphasized rather than dispelled by a short paper on *P. vivax* by Wolcott (22*c*). By applying the term "chromosome" to the compact masses of chromatin which other authors regard as whole nuclei the author contrives to see the division of the nucleus as a process of mitosis. But the four photographs offered in support of this thesis are scarcely adequate to establish this point and, besides, allow several other interpretations. It is certainly not being unduly fanciful to say that in size, range of shapes and in their staining properties the bars and rings of chromatin with their attending centrioles in Wolcott's illustrations bear an extraordinarily close resemblance to the corresponding structures in bacteria. This is obviously a field where much work remains to be done.

II. THE CHROMATIN STRUCTURES OF BACTERIA

A. Technique

1. Introductory Remarks

The student of bacterial cytology faces many difficulties. Bacteria are much smaller than the kinds of cells which have provided most of our knowledge of the structure of nuclei, and the details of the interior of many bacteria, including most of the cocci, are outside the area of useful employment of the light microscope. The parts of bacterial cells are not readily distinguished during life and in most instances standard techniques of fixation and staining fail to make them visible. Another peculiarity of bacteria, one affecting particularly their chromatin structures, wants emphasizing at the outset. It is the extent of the changes of internal organization which many kinds of cytologically rewarding and hence popular bacteria undergo in the course of their growth cycle—often in the span of a few hours—and in response to varied conditions of cultivation (27, 28).

The student who first looks at the colored drawings of growing and dividing cells of *Bacillus mycoides* which Guilliermond (29) published in 1908, with granules of chromatin haphazardly disposed in the lamellae of a much vacuolated cytoplasm, may find it difficult to believe that Badian's diagram of a few neat symmetrically placed "chromosomes" (30) in growing cells of the same bacillus can be equally true. And yet it is so. This *plasticity of shape* combined with a remarkable *uniformity of texture* is one of the outstanding properties of bacterial chromatin structures. It is as obvious in the older illustration as in the most recent electron micrographs of thin sections.

It is permissible to speak in this sweeping manner because the chromatin bodies of all bacteria that have been properly examined are very similar. All are instances of a single class of structures. The problem of the bacterial nucleus, which is the problem of its mode of division, can therefore be discussed without loss of generality on the basis of the findings in a few well studied species.

2. Observations on Living Cells

In most bacteria a nucleus is not conspicuous during life when they are examined in a microscope illuminated in the ordinary way. Under these conditions cocci and small rod-shaped bacteria look bright and homogeneous. But *Bacillus* species and relatively large enteric bacteria such as *Shigella dysenteriae*, examined during the first few hours of growth in fresh medium, are not entirely featureless. Bands of lighter gray may be seen in them which contrast with dark materials at the poles, along the sides, and across the middle of the bacteria. Phase contrast microscopy enhances these subtle differences and has securely established that the relatively transparent areas in growing bacteria correspond to the sites of the chromatin bodies of stained preparations (31–33). In favorable instances the correspondence between the living and the fixed image is close, as in the work of Stempen (32), but elsewhere the resolution of detail has proved uncertain. Earlier studies have now been eclipsed by the striking phase contrast photomicrographs of *Escherichia coli* and *Bacillus cereus* growing in media of high gelatine content recently published by Mason and Powelson (34). So far the main contribution of phase contrast microscopy to the study of bacterial chromatin structures has been the information it has given on the vague contours, the texture, dimensions, numbers, and arrangement of the chromatin bodies in living bacteria. The improved technique of Mason and Powelson will increase the usefulness of phase contrast microscopy for checking the effects of fixation procedures and the plausibility of published descriptions of the behavior of chromatin bodies.

Phase contrast microscopy is most successful with bacteria from young cultures. Optical differences within the cells seem to level off as the age of a culture increases. In certain species a further difficulty arises from the elaboration of droplets of lipid whose bright sheen blinds the observer to the subtle differences between chromatin structures and cytoplasm.

3. Fixation

Chromatin bodies can be demonstrated in bacteria killed by any one of many different methods such as drying in air, fixation with ethanol, methanol, Chabaud's solution, Bouin's solution, sublimate, picric-acetic acid, Perenyi's fixative, methanol formalin, potassium dichromate formalin, formalin, osmium tetroxide, and many others. Phase contrast microscopy has made

it clear that only very few of the many possible methods of fixation and staining give a true picture of the nature and behavior of the chromatin bodies. Among the good fixatives is Bouin's reagent, which preserves the shape, size, and arrangement of the chromatin bodies of certain bacteria (*Bacillus cereus*, *Escherichia coli*, *Shigella sonnei* and other enteric species) with remarkable fidelity (shown in figure 8). Under certain conditions good results are also obtained after fixation with osmium vapor. These two methods require brief comment.

Bouin fixation. Unfortunately (perhaps because of their excellent preservation) it has rarely been possible to obtain, directly or indirectly, a satisfactory *staining* of the chromatin structures in Bouin preparations (figure 8). A systematic analysis of this situation, as Cassel and Hutchinson (35) have recently emphasized, is needed—all the more so since it is apparently not difficult to stain the chromatin structures in Bouin-fixed cells of *anaerobic* gram negative bacteria (36, 37).

Osmium fixation. Osmium tetroxide, widely used and highly regarded in general cytology, and at present (as in 1912) the fixative of choice for studies of bacterial nuclei, preserves the shape of chromatin bodies less effectively than does Bouin's fluid but has the great advantage that it allows them to be brightly stained (after suitable pretreatment).

The results of fixation with osmium tetroxide must be interpreted with caution since it has been found that the usual length of exposure to osmium vapor leaves the chromatinic bodies of bacteria in a labile state in which they continue to respond, like those of living cells, with aggregation or fragmentation to changes in the concentration of sodium or potassium ions in the cytoplasm (38). In other words the results of fixation with osmium depend to some extent on the salt content of the culture medium at the moment of fixation and on the salt content of the fixative (if fluid) or that of any medium (agar, buffer) to which the fixed cells might be transferred after "fixation." It is probable that several published descriptions of chromatin bodies are based on unsuspected salt effects of this kind.

Cassel and Hutchinson (35) have pointed out that other fixatives besides Bouin's fluid have the property of leaving the chromatin bodies in an unstainable condition if drying is avoided during fixation, whereas good staining is possible when *dried* bacteria are treated with these fixatives. The authors have generalized these and other experiences into the statement that drying in air or with the aid of dehydrating agents is an essential step in all fixation procedures that leave bacterial chromatin bodies in a stainable condition. This is an exaggeration. Chromatin bodies of *Bacillus cereus* can be stained in the usual manner also after fixation through the agar in ways which avoid drying altogether, *e.g.*, with 3 parts of 1.5 per cent potassium dichromate plus 1 part of formalin. Several other examples of preservation of stainability after wet fixation, neither preceded by drying nor followed by treatment with alcohol, might be quoted, but this is not the place for technical details. The fact remains that there are many unexplained facts about the response of bacterial chromatin bodies to fixatives and stains; the experiments of Cassel and Hutchinson reveal this with greater clarity than earlier studies of chromatin bodies.

4. Staining

The chromatin bodies of bacteria seem to share some of the properties of the invisible germs of overwork in Sir Ralph's lament from Shaw's *The Doctor's Dilemma*: ". . . Some of them won't stain. They won't take cochineal, they won't take any methylene blue, they won't take gentian violet, they won't take any coloring matter!" There are important exceptions to almost any generalization about bacteria, but it is true that standard nuclear stains color bacterial chromatin bodies very poorly or not at all. The realization of this fact is implicit in the history of bacterial cytology; Dobell (40) complained of it long ago, and his experience has been confirmed many times.

Lately the emphasis has been more on the similarity between the staining properties of hydrolyzed bacterial chromatin bodies and hydrolyzed chromosomes of higher organisms, but this comparison remains lopsided so long as the *untreated* chromatin bodies' baffling failure to combine with common nuclear stains is not also remembered. Earlier observations on the lack of affinity of the "chromatin" bodies for aceto-carmine (31) and methyl green (41) have recently been confirmed by Cassel and Hutchinson (35) in systematic experiments with 3-hr-old cultures of *Bacillus cereus*. Hematoxylin and basic as well as acid fuchsin proved equally unavailing and this

has been also our own constant (unpublished) experience.

It adds to the difficulties of the situation that the staining properties of the chromatin bodies do not remain the same at all stages of the life of the bacteria. Thus Guilliermond (29), using iron hematoxylin, was unable to distinguish cytoplasm and chromatin in *Bacillus* cells from young cultures but easily succeeded in cells from cultures older than 8 hr. In several species the chromatin bodies of viable cells from old cultures can be stained directly with aceto-carmine although the same reagent fails to color them in cells from young cultures, leaving them silhouetted as blank spaces against the pink cytoplasm.

The influence of the composition of the medium is well illustrated by the experience of Duguid (42), who found that the chromatin bodies of *Aerobacter aerogenes* could be clearly differentiated from the cytoplasm after direct staining with methyl violet when the organism was grown on a medium containing sucrose and ammonium sulfate in the proportion of 1 to 0.01, but not when the proportions were 1 to 10.

Thus it would not be true to say that bacterial chromatin bodies can never be stained directly with simple nuclear dyes, but it is true that these dyes cannot be relied upon to give a useful result *at all stages of the growth cycle* and are useless for most of the time in most instances.

It is of interest in this connection that Belozersky (43) stated that there is no protamine or histone in bacterial nuclei and that chromatin bodies of *B. cereus* do in fact give a negative result (Dr. R. G. E. Murray, personal communication) when tested for histones by the method of Alfert and Geschwind (44). One is forced to conclude that bacterial nucleoprotein may be of unusual composition, an impression which derives some support from the observations of Chargaff and Saidel (45) on unusual properties of nucleoprotein from tubercle bacilli. The recent announcement from the laboratory of Dr. S. Spiegelman (45*a*) that he has succeeded in isolating bacterial nuclei intact from lipase-digested protoplasts raises hopes that the chemistry of these organelles will soon be fully known. In collaboration with Dr. Spiegelman a cytological and chemical analysis of protoplasts and the fractions that can be isolated from them is now being made by Dr. P. C. Fitz-James in our laboratory. Results to date suggest that Spiegelman's original description of isolated nuclei as closed vesicles will have to be modified.

In most instances where chromatin bodies have been revealed in bacteria it has been done by uncommon stains or by modifications of standard procedures. Three methods—one employing a mordant, one a cytochemical test and one relying on a complex mixture of uncertain action—have among them provided most of the evidence of bacterial nuclei discussed in modern writings. They are: Heidenhain's iron alum hematoxylin stain, the Giemsa stain, and the Feulgen procedure, in its original form or in some of its recent modifications. Significant features of these methods are set out in the following discussion:

Iron alum hematoxylin (IAH). This stain has been in use for the last 60 years in all branches of cytology. In studies of chromosomes it no longer takes the first place, but protozoologists still regard it as the most important stain for general purposes (46), and until recently IAH was the main tool in studies of nuclear behavior in fungi. Guilliermond (29) in an important study which will be referred to again later used IAH to stain chromatin material in *Bacillus mycoides* and *Bacillus megaterium*. Satisfactory correspondence between IAH staining and the sites of Feulgen positive material was established by Delaporte (1) in many bacteria, including the bacilli previously studied by Guilliermond. Robinow (47) noted a similar correspondence between the positive Feulgen reaction of the extruded nuclear mass of hydrolyzed bacterial spores and their selective affinity for IAH. The description of the nuclear structures of *Selenomonas* by Boskamp (48), recently confirmed by the writer (49), is another example of the successful use of IAH. The method suffers from the disadvantage that it does not work equally well at all stages of the growth cycle. IAH does not stain the chromatin of bacteria from cultures in the early stages of logarithmic growth, a phase in which, by other methods, the chromatin bodies are particularly distinct. Methods which stain these early stages well, *e.g.*, the Feulgen process, are obviously more useful, and iron hematoxylin is now rarely used in bacterial cytology. This does not diminish the value of the pioneer observations of Guilliermond (29) and Delaporte (1).

Giemsa's stain. It can be stated without reserve that the direct staining of bacterial chromatin and its differentiation from the cytoplasm is

achieved more readily by stains of the Romanowsky series than by the simple basic dyes of common histological practice. Compound stains derived from Romanowsky's experiments with mixtures of eosin and methylene blue were first used on bacteria soon after they had proved their worth in the diagnosis of malaria. Stains of this class, Giemsa's most often, revealed bright red bodies in a "blue, lilac, or pink" cytoplasm (40). Where the same species has been studied by different workers results have been consistently the same since the beginning of the century. Indeed, within its narrow limits, the work of Feinberg (50), Dobell (40), and Douglas and Distaso (3) anticipates the essence of the findings of Badian (30), Neumann (51), and Hartman and Payne (52). The high complexity of the Giemsa staining reagent, vividly described in a memorable passage by Baker (53), does not encourage chemical analysis of its mode of action, but the universal choice of Romanowsky stains for differential blood counts and the diagnosis of malaria finds a partial justification in the cytochemical work of Jacobson and Webb (54). These authors demonstrated that the bright cherry-red color which the (May-Gruenwald) Giemsa procedure imparts to resting nuclei is due to the presence in them of deoxyribonucleic acid and to the fact that ribonucleic acid-protein appears blue-black under the same conditions of fixation and staining.

To understand why Dobell and his contemporaries thought so highly of the Romanowsky stains, one wants to remember their enormous value to parasitologists. In the years before their introduction many doubted that malaria parasites had a nucleus at all. Simple basic stains had failed to provide unequivocal evidence of the presence of such an organ, and it was an event of the first importance when the Romanowsky dye-complexes first stained something in malaria plasmodia—something that was readily accepted as a nucleus—in brilliant shades of "carmine-violet" (55)[2]. It was (and still is, of course) the same with the bacteria. "Most of the ordinary cytological stains," complains Dobell (40), "*e.g.*, Delafield's hematoxylin, carmine, safranin etc., I have found unsuitable for bacteria. They—like most of the ordinary aniline derivatives—are liable to stain the whole cell uniformly, without differentiating the internal structure." Giemsa's stain gave a very different result. With the overwhelming evidence from leukocytes, plasmodia, and trypanosomes before them it is understandable that Dobell and his contemporaries should have regarded the ever-present red staining structures in bacteria as akin to nuclei. Nor do their modern successors argue from different premises (52).

The Feulgen test. Students of the nuclei of higher organisms are still debating whether a positive Feulgen reaction really reveals compounds of deoxypentose nucleic acid (DNA) at the site where they are in the living cell; or whether Feulgen positive material is perhaps rendered diffusible by certain fixatives and by the hydrolysis which is an integral part of the Feulgen procedure, and is moved from its original site to the structures in which we find it when the test has been completed (56).

The same uncertainty, enhanced by their peculiar staining properties, exists in work with bacteria in which, following the pioneer work of Delaporte (1), Stille (57), Piekarski (58), and others, Feulgen positive structures have often been demonstrated (figs. 2, 13).[3] But this does not lessen the heuristic value of the Feulgen test in comparative morphological studies. It is characteristic of nuclei and chromosomes that they give a positive Feulgen reaction. As long as there is no good reason to suppose that the positive Feulgen reaction of the chromatin bodies in bacteria is given by substances different from those which are responsible for the positive reaction of the nuclei of higher organisms the value of the test as

[2] In several plasmodia those materials that stain red with Giemsa are also those which give a positive Feulgen test. But a curious problem is presented by *Plasmodium elongatum*, whose nuclear structures, although Feulgen positive, have hardly any affinity for Giemsa's stain (21).

[3] Despite the importance attached to a positive Feulgen reaction there are few useful pictures of Feulgen preparations of bacteria in the modern literature (with the notable exception of the work of Piekarski), and one often hears that the positive reaction of bacterial chromatin bodies is too faint to be recorded in photographs. Following the practice of mycologists the writer has consistently obtained clear positive reactions in both gram positive and gram negative species after fixation with acetic acid-alcohol (3:1). As has been found in fungi, the color of the stain is deepened and its visibility is improved by mounting the treated specimens in aceto-carmine (59) (see figs. 2 and 13).

an indicator of a common nuclear component remains independent of changing chemical interpretations. Actually, there is good evidence from direct chemical determinations that the Feulgen positive material of spores contains DNA (60), and the loss of the affinity of chromatin bodies for direct Giemsa after treatment with deoxyribonuclease indicates the same for the chromatin in vegetative cells (61, 62).

Not long ago Atwood and Ornstein (63) discovered that a selective nuclear stain, brighter than standard Feulgen, is obtained when the fuchsin in the Schiff reagent is replaced by azure A or thionin. The introduction of this intense, yet delicate stain into bacterial cytology by DeLamater (64) has caused a marked improvement in the quality of published photomicrographs of bacterial chromatin bodies. For a critical assessment of the conclusions to be drawn from SO_2-thionin staining, see (65).

5. Special Methods

In studies of bacteria the methods discussed under *Staining* are used in the same way as in general cytology, but out of the Feulgen reaction have come several techniques designed to overcome special difficulties which one encounters in trying to stain bacterial chromatin bodies. These techniques have also proved very useful in cytological studies of blue-green algae and fungi.

The HCl-Giemsa technique and other devices for reducing cytoplasmic basophilia. Wishing to increase the brightness of the Feulgen stain in bacteria, Piekarski (58) followed it with Giemsa's stain. In control preparations in which hydrolyzed bacteria were stained with Giemsa solution without first being subjected to the Feulgen test, the chromatin bodies were brightly stained and more clearly differentiated from the cytoplasm than in unhydrolyzed Giemsa stained cells. The chemistry of this useful procedure is still problematic.

Giemsa's stain, as already mentioned, also stains chromatin bodies directly. It must now be admitted that this is more easily accomplished in gram negative than in gram positive bacteria and that even in the former good results are, at best, patchy. Neumann (51), who was particularly successful in the direct application of the stain, complains of "the treacherous Giemsa." The great advantage of a pretreatment with warm HCl is that it insures *uniformly* well stained preparations. Owing to the great reduction of cytoplasmic basophilia, there is in such preparations excellent contrast between nuclear elements and cytoplasm. Differentiation with 1 per cent eosin (30), ribonuclease (66), and perchloric acid (67) have been used to the same end, and the use of neutral salts, of alkali and detergents, has also been recommended.

However, the results of the action of ribonuclease and of N/HCl on fixed bacteria are apparently not entirely comparable. Both treatments remove ribonucleic acid from the cytoplasm (68), but the enzyme fails to raise the normally very low affinity of chromatin bodies for basic stains, whereas hydrolysis with N/1 HCl increases it very considerably. The chemical basis of this direct effect of N/HCl on the nuclei is not yet understood. But the interesting observation of Mudd and Smith (69) that hydrolyzed chromatin bodies have greater electron density than unhydrolyzed ones suggests the disquieting possibility that hydrolysis may cause material to shift from the cytoplasm to the nuclei.

There is satisfactory congruity between the sites of the positive Feulgen reaction and the areas that are stained reddish purple by Giemsa after hydrolysis, but the latter are invariably more extensive than the former and different materials are probably involved in the two responses. That an affinity for Giemsa after hydrolysis need not go hand in hand with a positive Feulgen test is well illustrated by the canoe-shaped parasporal body of *Bacillus laterosporus* (70) which stains brilliantly reddish-purple with Giemsa before and after acid hydrolysis but is Feulgen negative.

Techniques for making permanent preparations. Much useful information has been obtained from wet films of fixed and stained bacteria, but wet mounts are short-lived and many workers have felt the need to make some of their specimens permanent. To this end Delaporte (1) and Piekarski (58) used the conventional alcohol series followed by xylene and Canada balsam. Robinow (47), working mainly with the alcohol soluble Giemsa stain, used the standard method of dehydration by means of acetone-xylene mixtures with increasing xylene content. Both alcohol and acetone are liable to cause considerable shrinkage and several workers now prefer to make wet films

permanent by mounting them directly in the water soluble resin "abopon"[4] (71).

Superior results have been claimed for the technique of freeze-drying stained wet films of bacteria by long extraction with deep cooled absolute alcohol. This procedure, which the Philadelphia school has used routinely (often with excellent results) since its introduction by DeLamater in 1951, has been severely criticized by Bisset (72). DeLamater (73) has replied at length and has reasserted that "preparations handled by this technique have none of the distortion which occurs in routine dehydration procedures at room temperature . . ." One cannot help feeling that the real problem has been missed in these exchanges. It is not whether one method of dehydration is worse than another, but whether the debated technique demonstrably diminishes, preserves, or increases the amount of detail visible in wet films *before* dehydration. Robinow and Hannay (74) have made several experiments to find this out and concluded that, in their hands, the DeLamater procedure faithfully preserves, but does not increase, the fine detail already visible in wet mounts *of the same cells* before dehydration. They noted slight shrinkage of the bacilli and the disappearance of cell boundaries. It seems therefore that Bisset's criticisms are not justified, nor is DeLamater's claim that dehydration is essential to make fine detail clearly visible. It is proper staining more than any other factor which determines the quality of the detail that can be seen and photographed. To the reviewer it would seem that DeLamater's contribution to the arsenal of stains has been more helpful than the introduction of a useful, but not essential, method for the permanent preservation of stained specimens.

B. Results

1. Review of the Main Concepts of the Nature of Bacterial Chromatin Bodies

Having discussed some of the methods which have been important in demonstrating the existence of chromatin bodies in bacteria, we will now consider the evidence that has been collected with their help. Three major concepts can be distinguished:

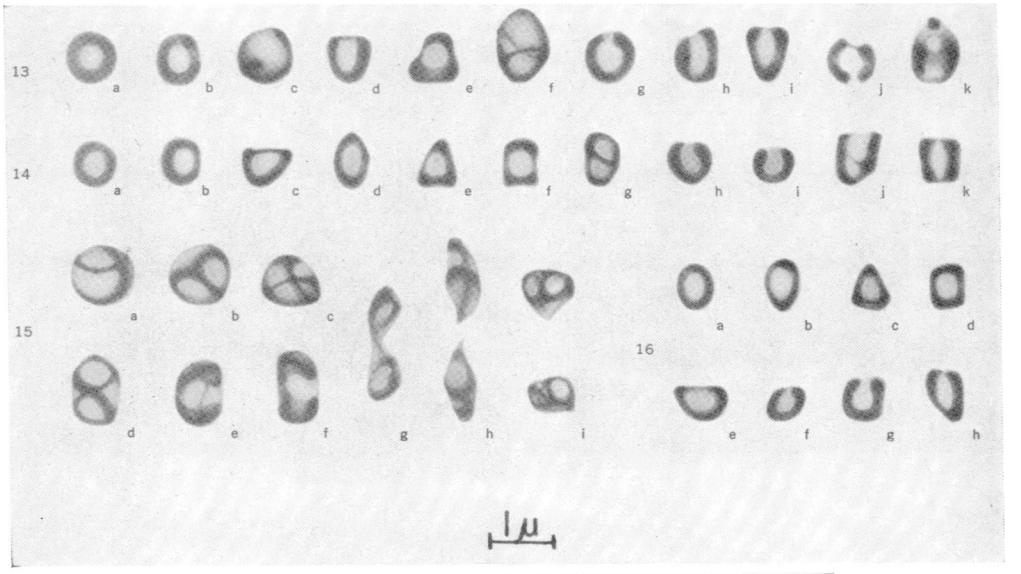
Until the early thirties the best documented observations converged on the picture of a simple, not visually differentiated lump of chromatinic material ("élément nucléaire," "filament axial") which divides directly and whose shape depends on the shape of the containing cell and the space available in it; in other words, a plastic entity with no characteristic shape of its own. A single round granule in cocci, a stout cord in rod forms with unobstructed cytoplasm, it would elsewhere assume the shape of a mass of "chromidial" (invisibly interconnected?) granules scattered between inclusions in the cytoplasm (figure 3). Clear, concordant illustrations of chromatin arranged in this irregular fashion are in papers by Guilliermond (29), Dobell (40), Douglas and Distaso (3), Schaede (75), in Delaporte's great memoir (1) and in a recent article by Robinow (76). Although the structures referred to have sometimes been spoken of as "nuclear" by their discoverers, they bear no resemblance to the chromosome-nuclei of general cytology described earlier in this article. A nucleus is an organization, never a "material." Dobell (40) and Delaporte (1) were both keenly aware of this fact, but elsewhere less critical attitudes prevailed.

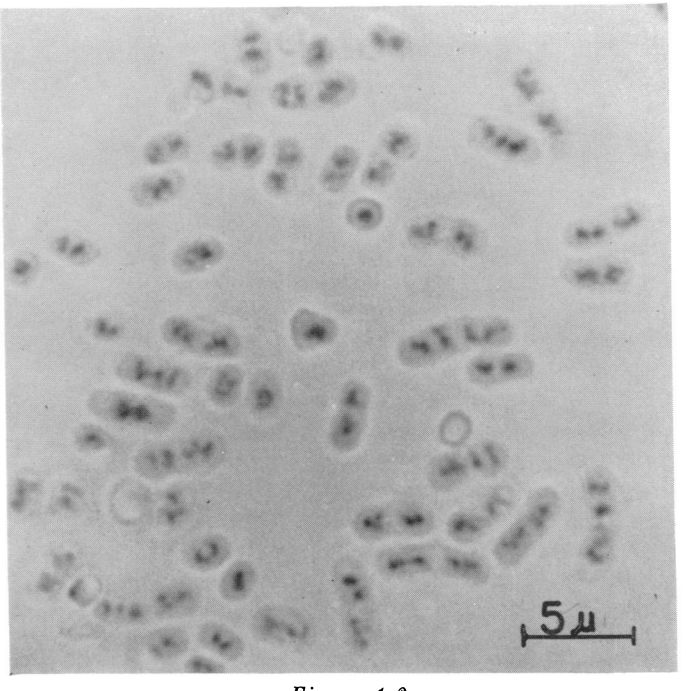
From 1953 onwards interest became centered on chromatin structures which corresponded more closely than the vague "nuclear material" of the preceding era to conventional ideas of what a (bacterial) nucleus ought to look like. Named "nucleoids" by Piekarski (58), these structures —simple, compact, round or composed of pairs of three or four granules or dumbbell-shaped elements—were remarkably constant in size, shape, and numbers. Their mode of division continued to be described as direct but with the refinement that the rodlet type was said to divide longitudinally, a feature which was lightheartedly accepted as indicating a relationship with chromosomes (30, 47, 77).

One need not despair of discovering a common bond between the shapeless "éléments nucléaires" and the neat "nucleoids"; indeed, it has been demonstrated by Delaporte (78), but there is no similarity between these two concepts and a third, more recent one, that bacteria have chromosome-nuclei and that these divide by a conventional process of mitosis. This provocative idea has been advanced in many papers by DeLamater and his associates since 1951.

The evidence which led to the formulation of the first two concepts will now be discussed in detail and an attempt will then be made to arrive at an idea of the nature of the chromatin organ

[4] Glyco Products Co., 26 Court St., Brooklyn, N. Y.





Figures 1-2

Figure 1 (top). Drawings of the chromatin bodies (*i.e.*, not obviously chromosomal "nuclei") of *Plasmodium elongatum*. Flemming, Feulgen. From Chen (21). (Originally published in the J. Infectious Diseases. We thank the copyright owner, the University of Chicago Press, for permission to reproduce.)

Figure 2 (bottom). Positive Feulgen reaction of the chromatin bodies in germinating spores and vegetative forms of *Bacillus megaterium*. Acetic acid-alcohol, Feulgen. Photographed in aceto-carmine. × 2700.

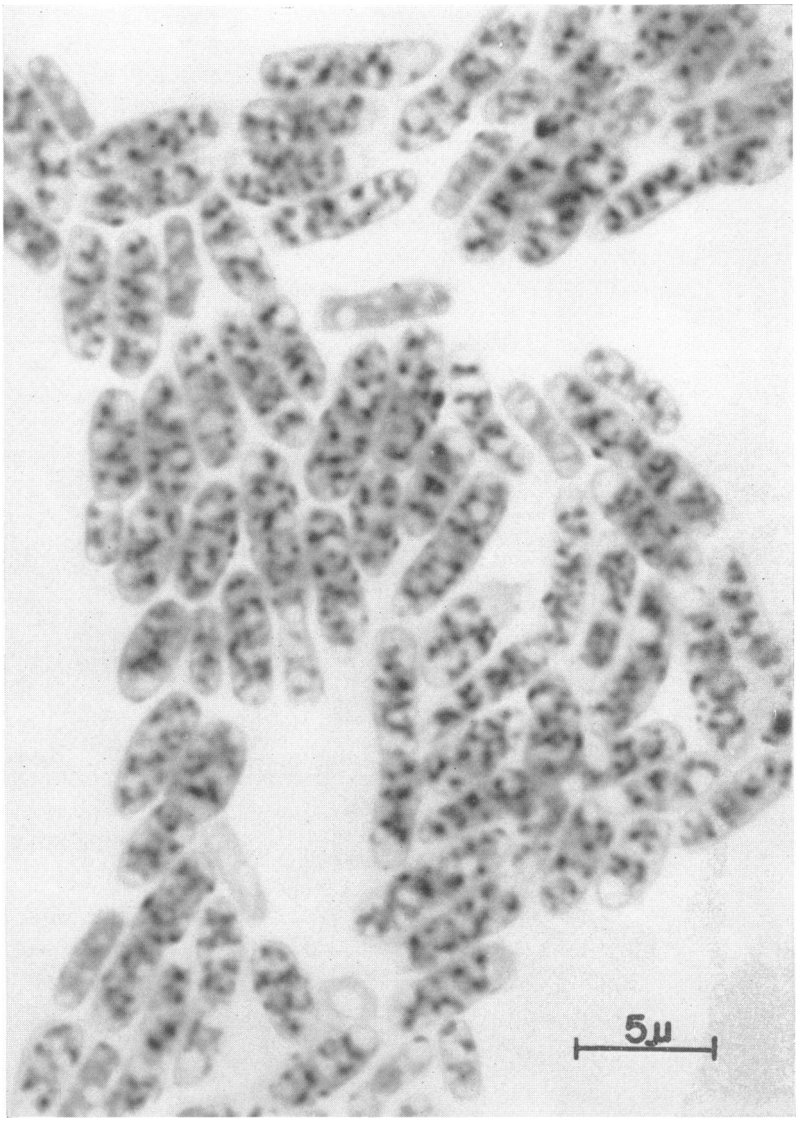


Figure 3

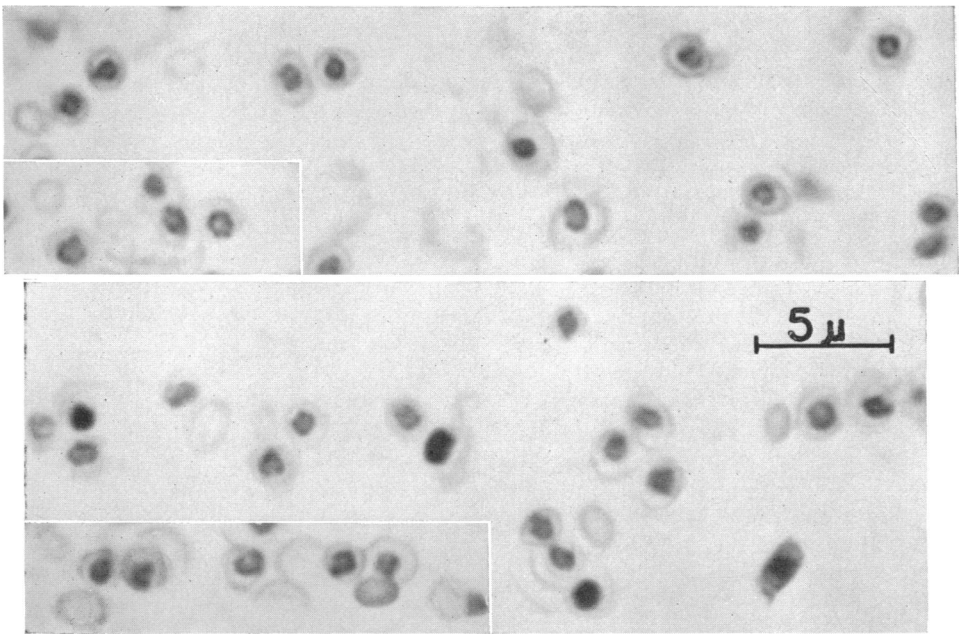


Figure 4

Figure 3. Bacillus megaterium. Bacilli from an 18-hr culture at about 20 C on yeast extract glucose agar. Fixed with osmium tetroxide vapor during the lag phase of renewed growth, 1.5 hr after transfer to fresh nutrient medium. HCl, azure A, SO_2 (Huebschman). × 3600. Numerous chromatin bodies are randomly dispersed in the "foamy" cytoplasm of bacilli laden with (unstained) lipid. This is a good example of what Guilliermond (29), speaking of *Bacillus mycoides* and *Bacillus megaterium*, has described as "agglomerations des granules colorables dans les neuds d'un cytoplasme alvéolaire."

Figure 4. Disposition of the chromatin in resting spores of *Bacillus megaterium* cracked open and rendered stainable by being shaken with glass beads while suspended in alcohol. Ribonuclease/Giemsa. × 3600. Preparation and microphotographs by Dr. P. C. Fitz-James. Details of his technique are given in J. Bacteriol., **66,** 312 (1953).

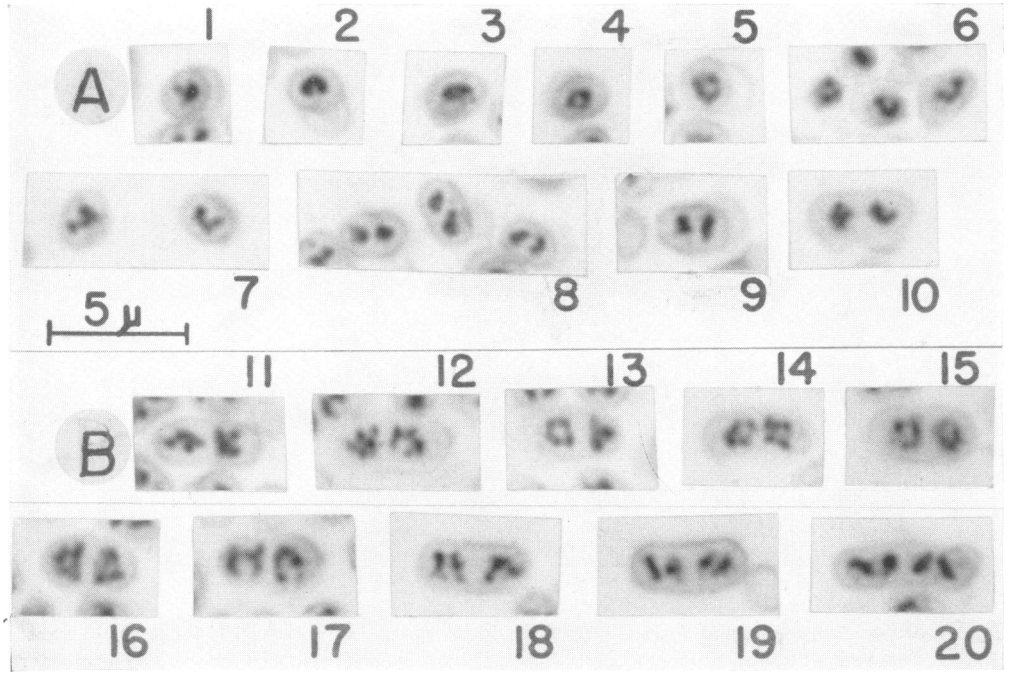
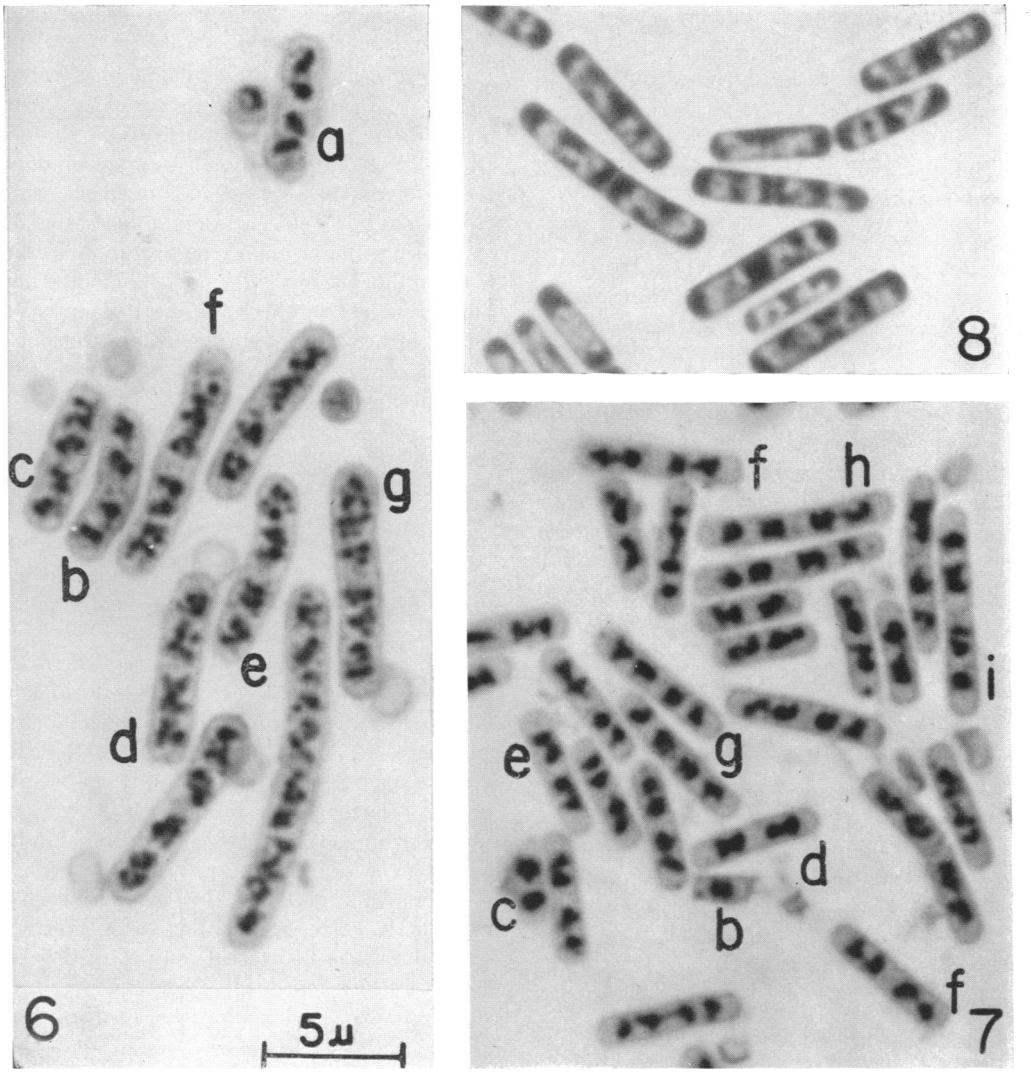


Figure 5. Successive stages of the first two divisions of the chromatin bodies in germinating spores of *Bacillus megaterium*. Division 1 begins at *A*5 and ends at *A*9; division 2 begins at *A*10 and ends at *B*18–19. Stages *B*12–16 are different aspects of the same half-way stage of the second division. OsO_4 vapor, HCl, DeLamater's SO_2-thionin, or Huebschman's azure A, SO_2. × 3600.



Figures 6–8

Figure 6. Successive stages of the third division of the chromatin bodies in a group of *Bacillus megaterium* cells (*a–g*) growing out of recently germinated spores. This photograph overlaps with and continues the sequence illustrated in the previous figure. The last stage of figure 5, bacillus *B*20, is identical with bacillus *a* in figure 6. × 3600. Originally published in: *Bacterial anatomy*, edited by E. T. C. Spooner and B. A. D. Stocker, Cambridge University Press, Cambridge, England, 1956. We thank the copyright owners, the Cambridge University Press, for permission to reproduce.

Figure 7. Bacillus cereus: 2.5 hr growth at 37 C on 2 per cent Difco tryptose 0.8 per cent NaCl agar. Killed *in situ* with OsO_4 vapor and transferred to glass without drying by postfixation through the agar with 70 per cent alcohol containing 1 per cent acetic acid. HCl, azure A, SO_2 (Huebschman). The habit of the chromatin bodies is typically "nucleoid." Stages *b–i* illustrate successive stages of growth and direct division of the chromatin bodies in bacteria of increasing length. Stage *a*, not represented here, corresponds to the spore with its single resting chromatin body. × 3600.

Figure 8. Bacillus cereus: 2.5 hr growth from an inoculum of spores on heart infusion agar at 37 C. Transferred to glass without drying by fixation through the agar with Bouin's mixture. Stained for 40 sec with 0.01 per cent toluidine blue in water. The chromatin bodies stand out, unstained, from the basophil cytoplasm. The geometry of the chromatin bodies in Bouin-fixed preparations is in good agreement with the information about chromatin bodies that has been obtained from electron microscopy. × 3600.

of bacteria. The evidence for mitosis will be discussed in a later section.

2. The Chromidial Nucleus and the Protean "Élément Nucléaire," 1908–1939

The achievement of this period is clearly and logically expressed in a memoir (1) of the French botanist Berthe Delaporte, whose work has not everywhere received the close attention which it deserves.

Delaporte's *Recherches cytologiques sur les bactéries et les cyanophycées* opens with a very complete, perhaps too charitably assembled catalogue of the literature on nuclei in bacteria from van Tieghem (1878) to Piekarski (1937). After the examination of more than 400 papers on bacterial cytology, Delaporte finds only one unifying feature in this welter of fitful, uncoordinated observations of generations of bacteriological Sunday cytologists. "Presque tous les auteurs," she remarks dryly, "admettent la présence de substance nucléaire individualisée mais la voient sous des formes très diverses." Some of the causes of this diversity were soon to be clarified by Delaporte's own extensive studies.

With the aid of iron alum hematoxylin-eosin and the Feulgen reaction, Delaporte examined the morphology of the chromatin in a wide variety of cocci, spirilla, a dozen different *Bacillus* species, *Azotobacter*, *Achromobacter*, chain-forming and single-celled sulfur bacteria, *Oscillospira*, and blue-green algae. No one else has since so carefully discussed the structure of so many different organisms in the pages of one single publication. Delaporte did not limit herself to the demonstration of chromatinic material. In extensive series of companion preparations she noted at the same time the amount and disposition of lipids, metachromatic granules and glycogen in the bacteria whose chromatin bodies she was studying. The results of her work are remarkable for width rather than depth. The range of shapes which the chromatinic material assumes in different bacteria is illustrated by numerous drawings of isolated cells assembled by the author into small uniform groups. The drawings bear the mark of unbiased observation and are entirely convincing, but they do not add up to life histories and they leave it to the reader to reconstruct the mode of division of chromatin bodies for himself.

These oddities of presentation do not detract from the weight of the general conclusions which Delaporte was able to draw from her many observations. She confirmed many of the older isolated observations on bacterial nuclei [*e.g.*, those of Dobell, 1911 (40)] and established beyond doubt that bacterial cells possess an organelle which gives a positive Feulgen reaction and stains like chromatin with iron alum hematoxylin. According to the author the nuclear organ of bacteria is very simple and not in the least like the nucleus in cells of higher organisms. The nuclear element of bacteria always has the same uniform texture. It divides by pulling apart and never gives rise to chromosomes. Its shape is variable and dependent on the shape of the cell which harbors it. When droplets of lipid are present, as they often are in the bacilli most frequently studied by cytologists, the chromatin is compressed into very irregular patterns, at times even fragmented into a mass of splinters and large and small granules, the "chromidia" of Guilliermond (29)[5] (see figure 3). In other kinds of bacteria the chromatin structures, according to Delaporte, are similarly deformed by droplets of sulfur or concretions of calcium carbonate. It is well known that the nuclei in some tissues of higher plants and animals assume bizarre shapes because they are compressed by unyielding materials elaborated in the cytoplasm or for other reasons. In these instances it is the shape of the *resting* nucleus which is irregular. In bacteria, according to Delaporte, it is the chromatin bodies not only of resting but also in *growing and dividing* bacteria which are liable to be stretched, compressed, or fragmented in various ways. Delaporte drew and described what she saw and did not try to make her findings conform to a preconceived way of nuclear behavior. In the eyes of her contemporaries the work

[5] In his valuable review, *The cytology of bacteria*, Lewis (2) acknowledges the correctness of Guilliermond's observations but asserts that the alleged "chromidia" are only bits of cytoplasm compressed between globules of lipid or volutin. This criticism overlooks the fact that Guilliermond was able with the aid of the iron alum hematoxylin-eosin double stain to distinguish chromatin from cytoplasm in the lamellae of compressed protoplasm between the "vacuoles" (*i.e.*, unstained lipid droplets). This distinction has been amply confirmed by the Feulgen preparations of Delaporte (1) and by observations of Robinow and Hannay (74) and of Robinow (76) on the disposition of chromatin in lipid-laden cells of *Bacillus megaterium*.

of Delaporte served only to relegate bacteria more firmly than ever to a class of primitive organisms fundamentally different from the rest of creation and comparable, at best, to the equally archaic blue-green algae.

Delaporte (78) has recently reinvestigated the disposition of chromatin in *Bacillus* cells and *Escherichia coli* with the help of the HCl-Giemsa method and has obtained much fresh evidence in support of her earlier conclusions. It should also be noted that Delaporte's views fit more harmoniously than other published concepts into the picture of the structure and behavior of chromatin bodies that is now beginning to emerge from the electron microscopy of thin sections of bacteria.

3. *Nucleoids and Chromatinic Dumbbell Bodies*

While Delaporte was doing the work discussed in the previous section, the first of a series of papers were published by Badian (30), Stille (57), Piekarski (58), Neumann (51), and others which were to determine the character of much of the bacterial cytology of the next 10 years. These studies had in common that they dealt with bacteria from *very young, growing cultures*, the very stages that had so often proved refractory to cytological examination in the past. The new findings, obtained largely with the HCl-Giemsa method, entirely reversed the existing situation by revealing that the nuclear structures of bacteria from young cultures, far from being inaccessible, are actually more readily intelligible than those in cells from older cultures. The "new" nuclei had none of the irregularities of shape, size, and arrangement characteristic of the "éléments nucléaires" described by Delaporte. The Feulgen positive "nucleoids" (nuclear equivalents) of Piekarski, also referred to as "chromosomes" by Badian or "chromatinic dumbbell bodies" or "chromosomes" by Robinow were neat little bodies whose arrangement exhibits a remarkable symmetry (figure 7). Most of them were found in configurations which looked like consecutive steps in an orderly maneuver of growth and division. Nuclear structures similar to those that were being rediscovered during the period under discussion had been described before in isolated publications by, among others, Nakanishi (79), Hoelling (80), Dobell (40), Douglas and Distaso (3), Paillot (81), and, closest in time, Stoughton (82*a*, *b*), but for a variety of reasons had not been pursued further by these authors or others.

The mode of division of the nucleoid bodies has been differently described by different authors. Hoelling's illustrations show directly duplicating elliptic discs, seen edgewise, with clear centers. Stoughton, Stille, Piekarski, Peshkov (83), Neumann and others saw them as round bodies which divide by elongation and constriction. Badian, Robinow, Klieneberger-Nobel (84), Flewett (85) and Bisset (86) speak of them as groups of short rodlets or slender dumbbells arranged, most often, at right angles to the long axis of the cell and dividing longitudinally through a series of intermediate Y- and V-shaped forms.

The new observations were soon confirmed in publications from many different laboratories. A consistent picture of the behavior of nuclear structures in bacteria began at last to emerge and the regular shape and constant numbers of the chromatinic bodies, as well as their orderly cycle of growth and multiplication, appealed to those who were looking for a visible embodiment of the mechanism for the duplication and distribution of heredity factors which, common sense demanded, bacteria must possess. The rise of bacterial genetics after 1947 made the idea of the nuclear nature of the chromatinic bodies even more plausible, and ushered in a new period of intense cytological activity. Unfortunately, understanding lagged behind technical progress, and it is now obvious that the work of the period 1933–1950 suffers from a serious imbalance of thought. In their excitement over the attractive and readily intelligible results of the newer methods the writers of the nucleoid school made only perfunctory reference (or none at all) to the work of earlier explorers. Between the lines of many a recent paper it can be read that the writings of Guilliermond, Dobell, or Delaporte, to mention only a few, are regarded as misguided and obsolete.

However, not everyone, at the beginning of the present era, regarded the nucleoids with uncritical enthusiasm. Schaede (75), looking for them in *Bacillus megaterium*, found only a disorderly array of Feulgen positive granules and expressed serious doubts regarding their alleged nuclear nature. His observations have never been disproved, but Schaede was sternly rebuked by Piekarski (87), and the discovery of the nucleoid bodies continued to be hailed not only as an

interesting observation—which it was—but as coming closer to the truth than the older concept of randomly distributed chromidia or cordlike "filaments axiales" which, as we shall see, it did not.

Convinced that it had been misguided, no one seems to have tried to *repeat the work of the earlier investigators (1900–1939)*. The effects of this emotional, unscientific, attitude are still felt in the bacterial cytology of today. The clue to the situation lies in differences of the *ages* of the cultures examined by different groups of observers. Schaede (75) did not find "nucleoids" in *B. megaterium* because he looked for them at a time when they are not there. Conversely Piekarski, Neumann, Robinow, and their followers did not trouble themselves about randomly distributed chromatin because they selected for study the one phase of the growth cycle in which the chromatin is tidily arranged in the shape of nucleoids and neglected the awkward later stages. In general, it may be said that most of the early observations of tidy, intelligible "nucleoids" have been made on preparations from very young cultures (30, 50, 51, 57, 58, 77, 87*a*), whereas haphazardly scattered unintelligible granules, chunks, and cords of chromatin have persistently been observed in preparations of the same kind of bacteria made from cultures past the exponential phase of growth (1, 3, 29, 40, 75, 76, 78). It might be reasonably objected that different fixatives have been used by different authors. This has proved to be unimportant. Not only did Dobell (40), Douglas and Distaso (3) and Schaede (75) actually use osmium tetroxide, the present-day fixative of choice, but also osmium-HCl-Giemsa or azure A-SO_2 preparations of cells containing comparable amounts of lipid, as Delaporte herself showed a few years ago (78); and more recently Robinow (76) in *B. megaterium* provided perfect replicas of many of the older illustrations of chromidial nuclei in quite differently fixed bacteria.

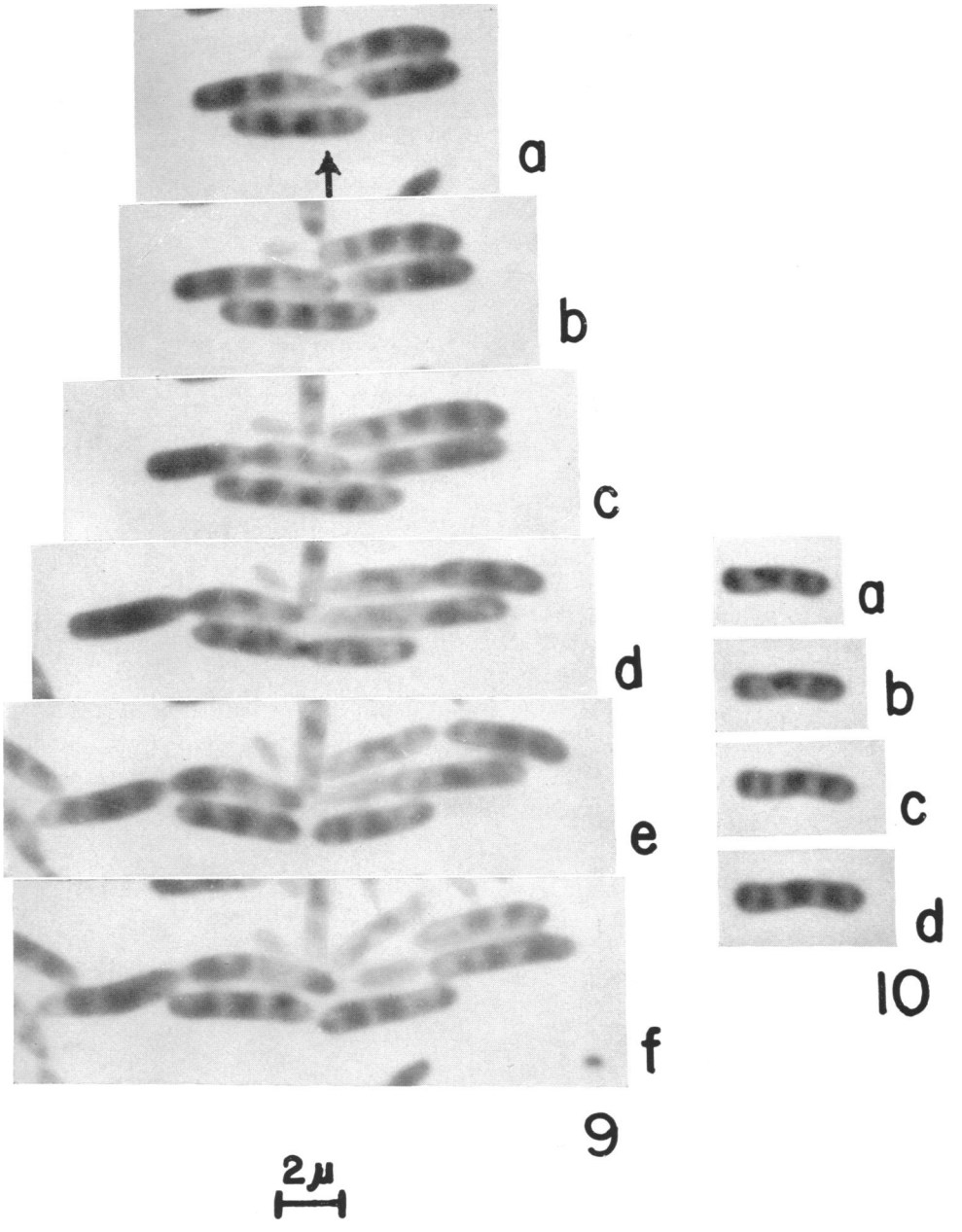
Recognition of the fact that the configuration of nuclear material in bacteria may change with age from order to disorder removes one of the main causes of a confusion of long standing. Dobell was right when he surmised in 1911: "My own belief is that the nucleus in *bacteria* may display not one, but many forms during the whole life cycle. Many of the nuclear structures which have been shown to exist in these organisms should, I think, be regarded as temporary states rather than as permanent conditions. The different results which have been reached by different workers when working, apparently, upon the same species, may to some extent find an explanation in this circumstance" (40).

4. The Mode of Multiplication of Bacterial Chromatin Bodies

General remarks. Before the chromatin bodies of bacteria can be compared with the nuclei of other protists it is necessary to find out how they divide. The relatively large cells found in very young cultures are more suitable for this purpose than the smaller cells of older cultures, especially of those species whose cytoplasm tends to get filled with lipid droplets and other inclusions which impress much confusing variation on the shape, size, arrangement, and numbers of the chromatin bodies of otherwise normal, viable cells.

The normal chromatin patterns in certain bacteria such as *Achromobacter fischeri* (88), *Azotobacter* sp. (89), some strains of *Shigella* (90), and the strange *Escherichia coli* strain C (27) are at all times complicated and often hardly intelligible; but even in more favorable species, such as the larger members of the genus *Bacillus*, it is by no means easy to see at a glance how the various types of constellations of chromatin bodies are related in time.

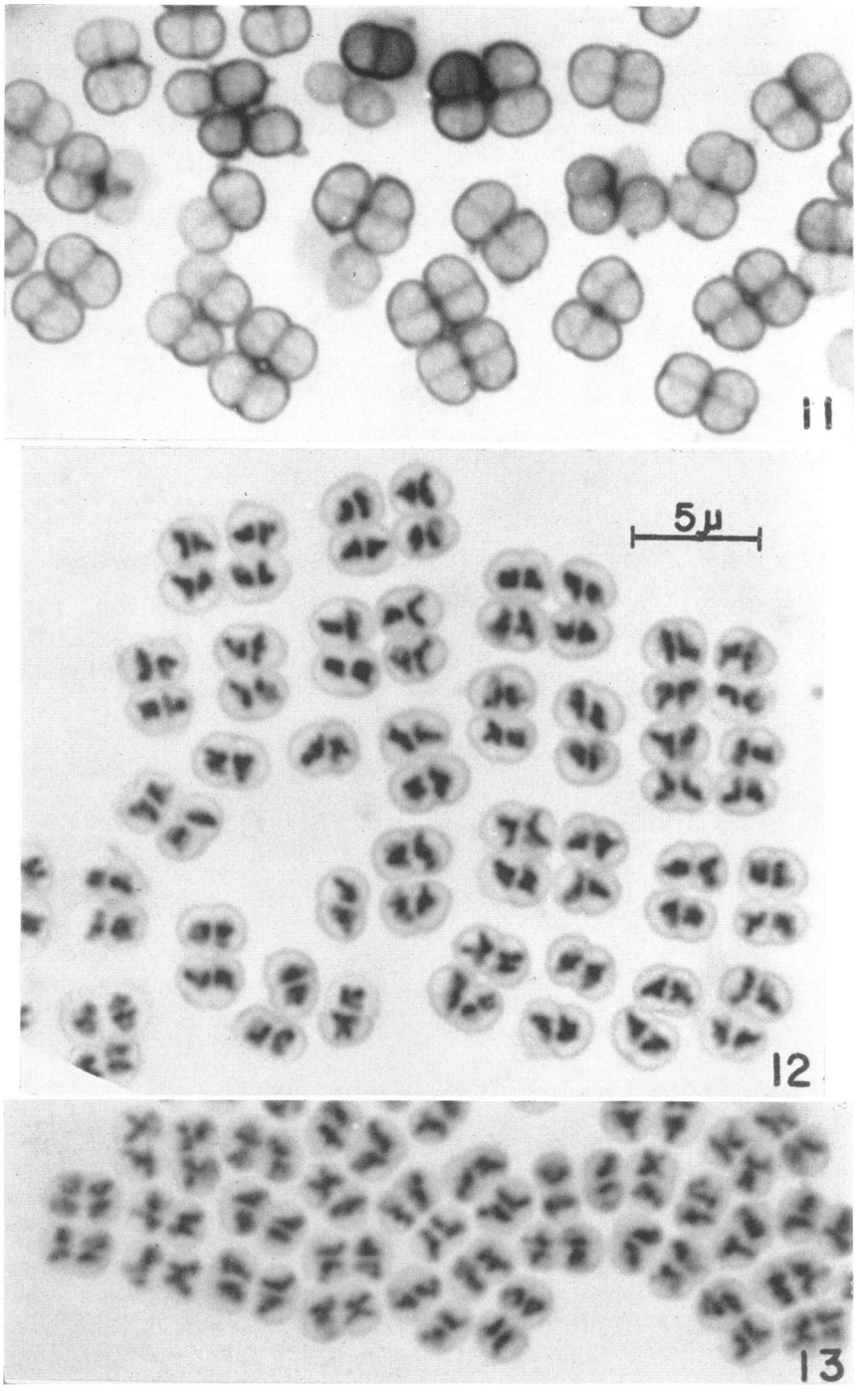
One difficulty arises from the presence of *several*, not necessarily synchronously dividing, chromatin structures in every growing bacillus, another from the fact that the chromatin constellations in any one preparation from a randomly growing culture in the exponential phase all look very much alike. Uncertainty deepens when good optical resolution increases the range of intermediate division stages [see, for example, Smith (91) on *Bacillus megaterium*]. Under these circumstances those trying to trace the course of division of bacterial chromatin bodies have been forced to construct their alleged division sequences with the aid of some objective indicator of the passage of time such as differences in length of the bacteria whose chromatin bodies they were comparing. The division schemes proposed by Stille (57), Piekarski (58) and Robinow (47, 77) were arrived at in this manner. But the sequences arranged by these authors were relatively short



Photographs of living growing cells of a species of *Achromobacter* taken at intervals of 5 to 7 minutes through a phase contrast microscope. (The unusually transparent bacterium was isolated from cow dung. Its probable identity was established by Dr. John Rishbeth of the Botany School, Cambridge University, to whom the author is much indebted for his kindness.) Chromatin bodies light, cytoplasm dark. *ca.* × 4000.

Figure 9. The single chromatin body indicated by an arrow in photograph *a* gives rise to two chromatin bodies in photograph *d* by what appears to be direct division.

Figure 10. The photographs illustrate how a single bar-shaped chromatin body, at the left end of the bacterium in (*a*) divides directly to give rise to two bar-shaped chromatin bodies in photographs *b*, *c* and *d*. Photographs taken by Mr. Roderic Pontefract.



Figures 11–13

and in the absence of information on changes with time in the relative frequencies of the different types described, inevitable in samples from randomly multiplying cultures, these early attempts can only be regarded as crude approximations to the truth.

The quantitative analysis of *synchronized* nuclear divisions in multinucleate bacteria, admirably carried out by Lark *et al.* (92), avoids these shortcomings and promises to become one of the most objective and informative means of studying the behavior of bacterial chromatin bodies.

Lark *et al.* started from multinucleate forms. But it is also possible, and technically easier, to study the mode of division of chromatin bodies by starting from a fixed point of reference in the shape of a uniform sample of single cells with only *one chromatin body in each*, for example, resting spores (figures 4, 5). The behavior of the chromatin bodies during the germination and early vegetative growth of the spore-cell may be followed in a series of samples taken at short intervals. To avoid confusion of successive generations the investigation should be limited to single bacilli with spore-cases still attached. Representative photographs of the cell types that follow one another after growth has started may then be arranged according to *length* or in sequences corresponding to *increasing numbers* of chromatin bodies, two characters which may or may not change at quite the same rate, but either of which indicates objectively the *direction* of the observed changes. There is genetic continuity between the members (types) of such a series, and provided the intervals between them are short and the series is long, it will come close to giving a true picture of the mode of division of the chromatin bodies. The usefulness of sequences of photomicrographs of this kind is obviously greatly increased when quantitative data on the relative frequencies of the different types of chromatin structures in cells fixed at different times after germination are also available for the same material.

Observations with the light microscope. Numerous observations have now made it plain that the chromatin figures in growing cells of many species of bacteria are very similar and fall into two categories: single solid shapes, and open complex ones. The solid shapes are straight or tapering bars or dumbbells, the open ones are clusters of three or four, probably connected, round granules of the same size as the halves of the dumbbells. These two categories are connected by intermediate forms such as dumbbells with one end nearly doubled, V-forms, paired dumbbells, H- and butterfly-shapes (76).[6]

[6] Delaporte formed the conviction that the chromatin in all rod-shaped bacteria normally assumes the shape of an axial cord. This is not borne out either by phase contrast microscopy or by the results of fixation with Bouin's solution or osmium tetroxide. It is true that axial filaments are commonly found in cells from regions of confluent growth of *Bacillus* species in cultures older than 5 to 7 hr, but a few minutes after the transfer of the containing bacteria to fresh nutrient medium the filaments are seen to "disaggregate" into several separate clusters of small chromatin bodies of the usual kind (Dr. R. G. E. Murray, unpublished data). In the presence of a sufficient concentration of salt, the reverse of this process, the aggregation of separate chromatin bodies into an axial cord, is equally readily induced in cells from

Figures 11–13

All figures are of preparations of a salmon-colored coccus which forms flat sheets of tetrads on agar. Recently isolated, it will shortly be described more fully elsewhere. The magnification is the same for all figures and is indicated by the scale in figure 12.

Figure 11. From 15-hr growth on meat digest agar at room temperature. Bouin fixation through the agar. Mordanted for 20 min with 5% tannic acid, stained for 5 min with 0.01% crystal violet. Photographed in water.

Figure 12. Fixed on the surface of agar with OsO_4 vapor and transferred to a coverslip without drying and with preservation of the natural arrangement of the cells. HCl, Azure A SO_2 (Huebschman), photographed in water.

Figure 13. Impression preparation on glass. Fixed in acetic acid alcohol after a few seconds drying in air. Feulgen. Photographed in aceto-carmine. Natural arrangement disrupted.

Details of the division of the chromatin bodies in the coccus remain to be worked out, but even now comparison of the chromatin bodies in figures 12 and 13 with those in *B. megaterium* (shown in figure 5 and figure 6) reveals considerable overall similarity. This is but one example of a remarkable uniformity which permits the making of wide generalizations about the chromatin bodies of bacteria.

A study of the first three cycles of growth and division of the chromatin bodies in germinating spores of *B. megaterium* has revealed that in this species the complex, open constellations are stages in the division of the compact ones (figs. 5, 6).

Direct observation of living, dividing chromatin bodies later showed this conclusion to hold also for a species of *Achromobacter* (shown in figures 9, 10). Single chromatin bodies first broadened, then became V-shaped and complex (three-granule stage) and thereafter divided directly into two single, narrow bars. Chromatin bodies closely resembling those in this bacterium are also found in many other species and genera. It seems reasonable to assume that they everywhere divide in the same simple, direct manner.[7]

young cultures by a sudden rise of the pH of the medium. There are reasons for believing that the formation of axial filaments is yet another example of the salt effects that result from (temporary) impairment of the cell's devices for maintaining a proper balance of sodium and potassium ions in its cytoplasm. Crowding, through a raising of the pH, may cause such an impairment and it is conceivable that the method of supravital staining with cresyl violet which provided Delaporte with most of her evidence is not entirely harmless and that the axial filaments which it demonstrates ought to be regarded as agonal artifacts.

[7] A division process of even greater simplicity has been described in *Salmonella typhimurium* by Lark *et al.* (92). But these authors were unaware of the fact, only recently established by Whitfield and Murray (90), that the chromatin bodies of bacteria tend to shrink and to suffer some aggregation in all situations which lead to an impairment of the mechanisms which maintain the proper balance of cations in the interior of the bacteria. Osmium fixation weakens these mechanisms without at the same time abolishing the sensitivity of the nuclear materials to changes in the ionic environment. In other words chromatin bodies of bacteria "fixed" by osmium tetroxide are quite as likely as those of living cells to change their shape in response to a change of the ion balance in their environment. The method of fixation used by the authors (which includes a "holding over" of fixed cells on salted agar), provides ample opportunity for such changes to occur. At present salt-induced aggregation must be regarded as the most likely explanation of the abnormally small size and uniform compactness of the chromatin structures in *S. typhimurium* preparations of Lark *et al.* If this source of error can be avoided, the study of synchronized divisions is bound to give results of very great usefulness.

Since the preceding paragraphs were written the direct division of the chromatin bodies of *E. coli* has been demonstrated in a series of remarkably detailed time-lapse phase contrast photomicrographs made by Mason and Powelson (34). It is obvious that their method of studying the events in living bacteria will make it increasingly unnecessary to rely on the kind of information from killed and stained specimens which has provided much of the material discussed in the present review.

Analysis of division maneuvers is difficult in bacteria such as *Shigella dysenteriae*, *E. coli*, strain C, and many others in which chromatin bodies tend to be arranged in complicated patterns. That the difference between the chromatin constellations in cytologically "easy" and "awkward" bacteria is one of degree and not of kind is shown by the fact that the chromatin structures of both consist of granules and rodlets of the same magnitude and behavior towards stains. Equally telling is the viability of the hybrids between "easy" and "awkward" bacteria which have been produced by Lieb *et al.* (93).

As described up to this point the chromatin bodies of the cytologically most favorable kinds of "growing bacteria from young cultures"—a limiting definition which cannot be repeated too often—appear to have the following properties: They are simple structures of relatively low density, not markedly basophilic but reacting positively in the Feulgen test. Normally they lie separately in the cytoplasm, and all those in one bacterium are homologous. Changes in the balance of ions in the cytoplasm may cause the aggregation of several chromatin bodies into a single continuous structure. This effect is reversible. Growth and division of chromatin bodies are attended by changes of form only, not by visible changes of texture (figures 6 to 8). In other words, there is no distinct resting stage. In the simplest type of body, that which in profile looks like a bar or dumbbell, division begins at one end and causes the successive appearance of V-, U-, and H-shaped phases. The chromatin structures of certain bacteria are netlike or spongelike, and their mode of division is not easily imagined. The problem here is of the same kind as that presented by the growth and division of the central, spongy chromatin structures in the cells of blue-green algae.

Information from electron microscopy. The electron microscopy of sectioned bacteria has confirmed the main points of the picture just out-

lined and has added details beyond the reach of the light microscope. The chromatin bodies in published electron micrographs occur in the same numbers, occupy the same fraction of the volume of the cell, and are arranged in the same manner as those in light optical preparations. The details of the division process are not easily reconstructed from randomly orientated sections of randomly multiplying chromatin bodies, but the V- and H-shaped profiles which have repeatedly been seen in thin sections of *E. coli* (28) and *S. typhi-murium* (94) may fairly be regarded as illustrations of the direct mode of division which had already been inferred from studies with the light microscope. The analysis of serial sections of bacteria, especially those of synchronously dividing cells (95) ought soon to clarify many points about the division of chromatin bodies which are at present uncertain.

Several different kinds of fine structure have been demonstrated in different sets of sections: Branching (hollow) cylinders of remarkably even caliber (95) and, most commonly, crisscrossing fibers of varying degrees of coarseness haphazardly disposed in a seemingly empty "vacuole" (96–99). At present the meaning of these formations is not clear. Identification of some of the fibrous structures with chromosomes (99) seems premature, especially since no fine-structure characteristic of chromosomes proper has yet been discovered. Factors known to affect the texture of chromatin bodies in thin sections include the salt content of both culture medium and fixative (Dr. R. G. E. Murray, private communication), and the nature of the imbedding material (96, 100); criteria for good fixation are still being sought.

Although the fine-structure of chromatin bodies in electron micrographs is still of dubious significance, these pictures already convey information of great interest. To begin with, coarse or fine, the structure of all the chromatin bodies of all the cells in a given section is usually remarkably uniform. This supports the conclusion from light microscopy that chromatin bodies divide without suffering obvious changes of texture, and lack a distinct resting stage. It is also already fairly obvious that chromatin bodies have a *fibrous structure*, a conclusion already arrived at by Stempen and Hutchinson (32*a*) from studies of the chromatin networks in "large bodies" of *Proteus* sp. Lastly, general agreement exists that the chromatin bodies in electron micrographs of sectioned bacteria lack a distinct envelope comparable to a nuclear membrane. It is significant that the very same techniques which fail to show a membrane at the surface of bacterial chromatin bodies invariably reveal well-defined membranes around the nuclei of other protists and of higher organisms. Obviously, if there is a membrane around chromatin bodies of bacteria it must be rather different from nuclear membranes commonly seen in other organisms.

"Cores" of chromatin bodies. To the concept of an open, fibrous chromatin body of unvarying texture but variable shape there must now be added a none too well defined component in the shape of a *nonchromatinic core*. With the aid of a special fixative Murray (39) has obtained evidence of Feulgen negative material, *distinct from the cytoplasm*, between dividing chromatin bodies of *Bacillus cereus*; and even in ordinary osmium-fixed material the nuclear structures of *Shigella dysenteriae* from the first hour of exponential growth have been described as composed of "chromatin granules connected by chromatin threads forming a reticulum which enmeshes and surrounds an achromatic 'core' " (90).

Other examples of cores associated with chromatin bodies are provided by the spores of *B. cereus* and *B. megaterium* in which the chromatin appears to be arranged as a simple or branched or beaded band around a spherical achromatic body (fig. 4). This configuration is seen in spores at an advanced stage of development (101), in mature resting spores (102, 103), and in spores in early stages of germination (101). The second division of the chromatin bodies in germinating spores of *B. megaterium* in particular leaves one with the impression that the dividing elements are arranged at the surface of a transparent spherical body. In *B. megaterium* such patterns are no longer common after the second or third post-germination division, perhaps because later there is much less space for the division maneuver of the chromatin bodies.

Disclike chromatin bodies with achromatic centers have on two occasions been described in the large *Oscillatoria*-like bacterium *Caryophanon latum* (104, 105), and the writer has recently discussed the possibility that the chromatin bodies of most other bacteria are also disclike though normally seen edgewise (76). Several chromatin bodies with nonchromatinic centers may be seen in fig. 12. The problem of the "cores" will no doubt soon be clarified by the study of thin sections.

Perhaps the most striking examples of cores in

chromatin bodies are to be found in cultures of enteric bacteria after short exposure to sublethal concentrations of "aureomycin" or chloramphenicol. Provided there is also present a sufficient concentration of sodium ions, the chromatin bodies of these bacteria will aggregate (temporarily) into the shape of a single sphere or chains of intercommunicating spheres in which a chromatinic shell is clearly distinct from a brightly refractile nonchromatinic core. It is disappointing to find that there is no difference in density or texture between cores and outer cytoplasm in the superb electron micrographs of thinly sectioned aureomycin-treated *E. coli* K12 recently published by Kellenberger and Ryter (100) despite the fact that in light-optical stained preparations the cores are quite different from the outer cytoplasm.

The "protrusions of cytoplasm into the central vacuole" (*i.e.*, the "chromatin body" of the present paper) which appear in Tomlin and May's (28) serial sections of *E. coli* are again not noticeably different from the cytoplasm surrounding the vacuoles. But the evidence for "cores" from electron microscopy is not entirely negative. Round masses slightly denser than the bulk of the cytoplasm have been found occluded in nuclear bodies of *B. cereus* (96), and in serial sections of *Bacillus thuringiensis* (a variant of *B. cereus*) similar inclusions have been seen to communicate with the outer cytoplasm through narrow channels (Dr. C. L. Hannay, unpublished data).

At present cores of chromatin bodies had perhaps best be regarded as regions of the cytoplasm that have become enclosed, probably only temporarily, in chromatin bodies and have through this proximity suffered a subtle change in their composition which expresses itself in stained preparations but is not always of a nature to make itself felt in electron micrographs.

If this were true one would expect occasionally to find similarly modified cytoplasm around the *outer* surface of chromatin bodies and this is, in fact, to be seen in many published electron micrographs of sectioned bacteria, *e.g.*, in figure 5 of Tomlin and May (28).

5. *Accessory Chromatin Granules*

DeLamater and Hunter (106) and DeLamater and Mudd (107) have discovered that the ordinary chromatin bodies in vegetative cells of *Bacillus megaterium* are often accompanied by very small round or oblong granules ("centrioles") which keep close to their larger fellows and like them are Feulgen positive. Their presence in *B. megaterium* has been confirmed by Robinow (76) and they have also been seen in *Bacillus cereus*. In *B. megaterium* they are most distinct in a fair proportion of germinating spores fixed before the end of the second division of the chromatin bodies. They are less easily found among the many closely packed chromatin bodies in fully grown vegetative forms of *B. megaterium* and *B. cereus*, but become conspicuous once more when the chromatin bodies have been induced to aggregate into large complexes by manipulation of the balance of ions in the environment. The most direct way of doing this is to transfer growing bacilli from a medium of low salt content to one of higher salt content. During the brief interval of adjustment to life on the new medium the chromatin bodies of *B. cereus* (in experiments of R. G. E. Murray) were found to have aggregated in the center of the cell and to have left their accessory granules behind in the more distal portions of the bacilli where they were now plainly visible. In other experiments (108) in which the efficiency of the "salt pumps" had been impaired by the presence of sublethal concentrations of tetracycline antibiotics or other reagents, the accessory granules have been found close to the central aggregate in arrangements resembling chromosomes in metaphase between a pair of centrioles. The origin of this artefact from the fusion of several independent chromatin constellations and the readiness with which it breaks up again when normal conditions are restored makes it extremely unlikely that this resemblance is more than purely geometrical. Some of DeLamater's photomicrographs suggest that the centrioles are capable of division. It is not yet known with certainty whether in normal cells they divide together with or independently of the chromatin bodies and whether they perform any function in the division maneuvers of the latter. As far as this reviewer is aware "centrioles" have not yet been identified in electron micrographs. No helpful interpretation of the nature of these intriguing structures can be advanced at present.

6. *Variability of the Shapes of Chromatin Bodies*

This discussion has dealt at some length with the chromatin bodies in cells from young cultures

because, in many species, their behavior at this stage of growth is readily intelligible. However, this type of chromatin body is but one of many different forms which the chromatin organ of viable, growing bacteria may assume. The chromatin bodies of *Escherichia coli* growing in a synthetic medium look different from those in cells of the same species multiplying in broth (27, 28). Even in the same medium the aging of a culture is, in many instances, *without loss of viability*, attended by striking changes in the morphology of the chromatin. As has been already indicated, the chromatin may be in the shape of an "axial filament," crumbs of chromatin of different sizes may be scattered through the cells, or there may be only one or two dense lumps of it, compressed between lipid granules (29, 1, 76). How the irregular chromatin figures grow and divide is not easy to tell. Under the light microscope all these shapes appear to have the same texture and all of them, through fusion of (seemingly) separate fragments or conversely by the disaggregation of large complexes, readily give rise to chromatin bodies of the ordinary type when the bacteria containing them are transferred to and begin to divide in a fresh nutrient medium. The open structure and uniform texture of chromatin bodies apparently permits the existence of an extremely wide range of viable arrangements which has no parallel among the chromosome-nuclei of other protists and the higher organisms.

7. *The Chromatin Structures in Bacterial Spores*

The most interesting aspect of spores, as Knaysi (109) has aptly remarked, is the story of their *development* but no systematic account of this process intelligible to cytologists has yet appeared. The matter has been briefly discussed by the writer in another review (76). Interesting electron micrographs of thin sections of *Bacillus cereus* and *Bacillus megaterium* engaged in the process of forming spores have recently been published by Chapman (110). The discovery that in *Bacillus thuringiensis* a crystal of protein arises in the cytoplasm of each sporing cell, and the demonstration of other types of "para-sporal bodies" in other species (111, 112) has resulted in an acute need for knowledge of the events leading to spore formation.

Something is known about the chromatin in *mature* spores. A clear view of the chromatin has been obtained in stained preparations of spores cracked, but not shattered, by bombardment with ballotini glass beads in alcohol (103) and in stained sections of spores (102). These studies have shown that: "In the spores of both *B. cereus* and *B. megaterium*, the chromatinic material is arranged in a ring, or variation thereof, composed of at least three segments. Although in a few cases these segments seem so closely associated as to form an unbroken circular band, the majority are arranged more loosely in a beaded ring. Many of these latter structures are broken at one particular point, possibly as a result of the disintegration. One notes also triangular forms, and in some three radiating arms form a clawlike structure. In a few, S-shaped and figure of eight patterns are encountered" (103) (fig. 4).

Electron microscopy of sectioned spores has disposed of certain erroneous published notions of the reviewer concerning the *arrangement* of the chromatin (113), but it has so far failed to give useful information on its *fine-structure* in *untreated* resting spores.

The extrusion of the chromatin to the exterior during Feulgen-hydrolysis of resting spores has enabled Fitz-James (60) to give the first direct chemical demonstration that bacterial chromatin contains deoxyribonucleic acid.

It was stated earlier that rings of chromatin, V-forms, and clusters of three or four granules must be regarded as stages of the division of unit chromatin bodies. This view does not go well with the fact that chromatin constellations of just this appearance are commonly found in mature *resting* spores whose nuclei one would certainly not expect to find in a state of division.

This dilemma is at present not easily resolved. One solution would be to allow that "three-granule clusters" and similar complex chromatin configurations in resting spores and vegetative cells are after all resting nuclei containing three simple chromosomes, as has been maintained by DeLamater and Mudd (107) and, in a different way, by Fitz-James (114). Tempting and plausible as it admittedly appears, this hypothesis is not borne out by the mode of division of the chromatin bodies that can be inferred from sequences of closely spaced, stained preparations of germinating spores of *B. megaterium*, where the reverse seems to be true. This concept is also contradicted by observations on the division of chromatin bodies in living cells of a species of *Achromobacter*, where,

again, it is only the *dividing* bodies which appear to be complex.

An alternative explanation would be to regard the open chromatin complexes in resting spores as being due to the elaboration of a relatively large "core" within the arms of an unusually branched chromatin body. Here, as elsewhere, time and the electron microscope will tell.

8. *Comparison of the Chromatin Bodies of Bacteria with Chromosome Nuclei*

The evidence which we have surveyed now permits us to make the following general statements: The chromatin bodies of bacteria lie separately in the cytoplasm. They contain Feulgen positive material but are not markedly basophilic either between or during divisions. Chromatin bodies lack a demonstrable membrane, have the same texture and staining properties between and during divisions, and divide directly in the sense that they are what they are at all times and do not, like chromosome-nuclei, pass through phases where they are something different.[8] Under certain abnormal conditions individual, dividing chromatin bodies readily fuse with their fellows in the same cell and as readily disaggregate after the bacteria containing them have been returned to a normal environment.

It is clear from this description that chromatin bodies are not comparable to the chromosome nuclei of higher organisms and of protists described at the beginning of this review.

9. *Affinities*

Do the chromatin bodies of bacteria have features in common with the chromatin structures of any other protists? Formally, at any rate, as Delaporte (1) has noted already, there is a great similarity between the chromatin bodies of bacteria and those in several kinds of blue-green algae (25). The central Feulgen positive, sponge-like body in the cells of these organisms consists of a maze of chromatinic threads and granules which often bear some resemblance to chromosomes. But there is about them no constancy of shapes and numbers, and the central organ divides directly in a manner of which it is still impossible to give a point-to-point description but which is certainly not a form of mitosis. Blue-green algae thus share with bacteria the distinction of having nonchromosomal chromatin organs. It remains to be seen whether closer inspection (in thin sections) reveals true affinity beneath the formal resemblance. Such observations would contribute fresh arguments to the discussion of the relationship of the blue-green algae and bacteria recently reconsidered by Pringsheim (115) and Stanier (116).

The writer knows of no other protists, besides the blue-green algae, with nuclei resembling the chromatin bodies of bacteria, though it is probable that they exist. A formal and perhaps misleading similarity between chromatin bodies and the nuclei of certain *Sporozoa* has been pointed out elsewhere (76)[9].

[8] Things of the size of chromatin bodies must consist of millions of molecules. It is also obvious that even above the level of molecules chromatin bodies must be more complicated than they appear to be in the light microscope. But this kind of complexity does not turn them into a "complex nuclear apparatus" (28). The difference between chromatin bodies and ordinary nuclei lies in this: that the latter, on dividing, give rise to sets of physically independent, complex individuals (chromosomes), each of which goes through its own cycle of division, whereas a chromatin body divides directly into two chromatin bodies. The geometry of division is thus very different in the two instances.

[9] This similarity exists between the range of shapes of chromatin bodies, in *Bacillus* species, to go no further, and the forms assumed by the nucleus of *Plasmodium elongatum* as described by Chen (21). Like the former, the "nucleus" of this parasite is a simple body with a nonchromatinic core and divides directly without giving rise to chromosomes.

That this coincidence may not be entirely fortuitous is suggested by the strong resemblance between the shapes assumed by this same nucleus and the shapes of the (much smaller) organisms of agalactia (pleuropneumonia of sheep) in the beautifully clear electron micrographs published by Klieneberger-Nobel and Cuckow (117).

It might be objected that "nuclei" of one species ought not to be compared with whole organisms of another species. However, in this instance it is, perhaps, permissible to do so. Bacteria are capable of giving rise to pleuropneumonia-like phases and, in one case at least, these "formes naines" have been described as being very nearly naked chromatin bodies (61).

It fits into this web of dimly perceived relationships that some phases of yet another sporozoon, *Theileria*, also resemble one kind of pleuropneumonia organism, namely that of agalactia of goats.

10. Concluding Remarks

At the close of this review we turn once more to its beginning. There it was pointed out that many protists have chromosome-nuclei which divide by mitosis, but that in the physiologically equally efficient "nuclei" of many other protists chromosomes are not obvious. It was also explained that such nuclei grow in a manner which is still obscure and that they divide directly. It now appears that among these must also be counted the chromatin bodies of bacteria.

C. On Claims of Mitosis in Bacteria

In the preceding pages evidence has been presented that, contrary to common belief, there is a good deal of continuity between older and more recent views on bacterial nuclei. Once it is recognized that the chromatin bodies in cells from old cultures may be arranged very differently from those in very young cultures, then the views of the era preceding and culminating in the work of Delaporte (1) are seen to be in good accord with those of the newer "nucleoid" school. It has also become evident that the picture of the nature and behavior of chromatin bodies that has emerged from observations on living bacteria, and to a larger extent on fixed and stained bacteria, is compatible, to a most encouraging degree, with the first exciting results of the electron microscopy of thin sections.

We must now turn to the evidence for the hypothesis of DeLamater that bacterial chromatin bodies divide by mitosis (106, 107, 118). Since it has been explained elsewhere in detail that the evidence which DeLamater has assembled does not prove his hypothesis (76), it will be permissible to deal with this matter more briefly in the present review.

DeLamater's extensive work, if I have read it correctly, is based on the thesis that bacteria must have mitosis because they continue to show, through indefinite series of divisions, the features characteristic of their kind. Knowledge of the behavior of the nuclear structures of blue-green algae and many other protists, which enjoy heredity without mitosis, makes this thesis untenable. However, while it is improbable that bacteria have mitosis (considering their nearness to the blue-green algae which lack it), it is clearly not *impossible* and the question can only be decided by observation.

The case for mitosis rests on photomicrographs of fixed and stained preparations of well-known bacteria. Most of the photographs are remarkably clear and rich in detail. The chromatin structures which they show have been found reproducible and are quite typical of the bacteria concerned. In the opinion of the reviewer there is little to criticize in DeLamater's methods of fixation and staining; indeed, we have all profited from them. Even if it were justified, criticism of technical matters would be entirely peripheral to the main issue, which is that of the author's standards of validity.

DeLamater's basic observations were made on multinucleate bacilli from randomly multiplying cultures. Alleged phases of the division of the chromatin bodies, such as "resting nuclei," early and late "prophase," "metaphase," "telophase," and "anaphase" were identified solely on the basis of their fancied resemblance to the corresponding phases of mitosis in the more familiar nuclei of higher plants and animals. No correlation with the growth of the containing bacilli was attemped. Unfortunately, bacterial chromatin bodies lack distinctive resting stages, and DeLamater has been forced to ascribe to them interphase nuclei of an uncommon kind, nuclei, namely, with few chromosomes which remain separately visible as beaded threads during the interphase. This device has enabled him to overcome the difficulty caused by the uniform chromaticity of all phases of the division of chromatin bodies. In short, DeLamater deals with the manifold variety of constellations of chromatin bodies in a randomly dividing culture as one might deal with a picture puzzle.

Now, there are two kinds of picture puzzles, one in which all or most of the pieces are different and another in which they are all alike. Let us consider the second kind first. This type of puzzle can only be solved when its plan is known in advance (*e.g.*, from the picture on the box containing it) or when the right solution can be inferred because it is the most plausible one. The solution of this type of puzzle is impossible when the plan is not given and when the criterion of plausibility does not apply, as in an abstract design.

It seems to the writer that DeLamater's method of selecting his illustrations is similar to that by which one solves a picture puzzle of the kind just described. To him all the pieces (chromatin constellations) appear interchangeable, and he arrives at the puzzle's solution by arranging

them in the most plausible manner. But, and this is the crucial point, the arrangement which he proposes is plausible only if it is taken for granted at the start that bacteria have mitosis. However, in truth, there is no picture of mitosis on the lid of this puzzle; the facts of general cytology make it impossible to take mitosis in bacteria for granted, its occurrence in bacteria is a mere assumption which remains to be proved. How can it be proved? Mitosis is a process in time. Short of observing it taking place in living cells, mitosis in bacteria can only be accepted if it emerges as the mode of division of the chromatin bodies in series of types of bacteria whose *relationship in time* has been determined with the aid of objective criteria *independent* of nuclear behavior.

When this has been grasped, then it is easily seen that a nuclear preparation of randomly dividing bacteria is indeed comparable to a puzzle, but to one of the first mentioned kind, that where all pieces (whole bacteria) are different (have different lengths) and where the position of each piece in the finished picture (the true time sequence of nuclear changes) is objectively determined by the spatial relation (time relationship) of each piece to its neighbor and not by the colored patches (chromatin bodies) printed on it. In other words: a preparation of this kind should be regarded as a puzzle of bacterial forms (length classes) and not of chromatin figures. When the time relationship of the bacterial forms has been solved, the time sequence of nuclear changes falls automatically into our hands. Lark *et al.* (92) and Birch-Andersen (95) are the first to have carried out experiments of this kind. The sequences which they were able to reconstruct from preparations of synchronously growing and dividing bacteria strongly suggest a direct mode of division of the chromatin bodies.

Sequences reconstructed from samples of germinating (uninucleate) spores fixed at intervals of a few minutes (76) likewise show that chromatin bodies divide directly and that the terminology of classical mitosis is out of place in describing their behavior. Synchronization of cell division in *Bacillus megaterium* by a brief lowering of the temperature of incubation has been achieved by Hunter-Szybalska *et al.* (119). Perhaps this elegant method will eventually provide us with a closely spaced series of cell types demonstrably related in time and revealing, step by step, the process of growth and division of the chromatin bodies. The pictures which illustrate the authors' first account of their experiments with this method, however, do not provide this information. They state that after chilling to 12 C, "almost all of the nuclei were in the phases of nuclear division characterized by configurations simulating metaphase and early anaphase." This description is misleading. The illustrations show that the chromatin bodies in the chilled cells have aggregated into arrangements which perhaps resemble metaphase plates of nuclear divisions *of higher plants and animals* but which are unlike any chromatin constellation normally seen in randomly multiplying *B. megaterium* (growing at 37 *or at 15 C*). In other words the chromatin bodies cannot properly be said to have been "arrested" at any phase of their normal cycle of division.

Return to warmth induces division or gradual "disaggregation" (38) of the chromatin masses. Only the beginning of the first cycle of resumed divisions is illustrated. It is clear that considerable synchronization of nuclear events has been achieved. But the series starts with an abnormal configuration and the fate of the awakened chromatin has not been followed in a sufficiently large number of sufficiently small steps to permit a reconstruction of the normal cycle of division.

DeLamater has sought to strengthen the case for mitosis with photographs of the aggregates of chromatin bodies which can be induced by exposing the containing bacteria to sublethal concentrations of tetracycline antibiotics and a variety of other chemicals (120). He interprets these aggregates as nuclei "arrested at metaphase" and such like, but the clear demonstration of their origin from the fusion of *several, separate, homologous*, chromatin bodies already twice given by Kellenberger and again by Whitfield and Murray (38) makes this interpretation untenable. Not only is the terminology of mitosis inappropriate for the description of temporary aggregates of irregular numbers of homologous chromatin bodies, but also it is known that the observed abnormalities are not caused primarily by a poisoning of the mitotic machinery but are in reality the result of a temporary imbalance of sodium ions in the cytoplasm caused by an impairment of the efficiency of the cells' "salt pumps" (37, 95). In media containing concentrations of salt far below those usually added to ordinary laboratory media, aureomycin, to give but one example, ceases to act as a "mitotic

poison"; in other words, no aggregates of chromatin bodies are found under these conditions. To find evidence for mitosis in these experiments is again possible only when unproved assumptions are made at the start and when the origin and prospective fate of the structures concerned are not taken into account.

D. Mitosis in Cocci

The study of the chromatin bodies of the cocci is hampered by two difficulties. The first of these is the small size of many of the common species such as the staphylococci which have lately been closely examined (120*a*, *b*). Here the chromatin bodies are so minute that few cytologists would probably care to say much about them and most would agree that their behavior cannot reasonably be described in terms of nuclei, chromosomes, mitosis, or meiosis.

The other difficulty arises from the spherical shape of many cocci and the habit, common among them, of having the planes of successive cell divisions displaced toward each other by a 90° angle (fig. 11). Add to this that multiplication of the chromatin bodies may be one or two steps ahead of cell division and it becomes clear that where the chromatin structures are large enough for rewarding microscopy as in the examples shown in figures 12 and 13, their division maneuvers can be grasped only by strict attention to the disposition of cell boundaries, preferably in undisturbed aggregates.

The literature on the chromatin bodies of cocci reflects the two kinds of difficulties outlined above. Many students of species with relatively small cells, from Dobell (40) and Delaporte (1) to recent times (121, 87a, 122) have been content to state that cocci have more or less spherical chromatin bodies, mere dots and spots of chromatin, which seem to divide directly. Electron micrographs of dividing chromatin bodies in *Staphylococcus aureus* (97) and in *Neisseria* (123) support this conclusion.

More ambitious claims have been made for larger species. In one of these, *Micrococcus cryophilus*, DeLamater and Woodburn (124) have seen what they believe to be evidence of mitosis. Their work has been severely, and I think justly, criticized by Bisset (122) who maintains that the authors have misinterpreted their results by failing to correlate the behavior of the chromatin bodies with the disposition of boundaries across the cells containing them. Bisset's own findings in the same organism again suggest a direct mode of division.

Recently the occurrence of mitosis and indeed meiotic mitosis in *M. cryophilus* has been reaffirmed by Hunter (125). Technically there is little to criticize here. Some of the photomicrographs illustrating this work are remarkably well resolved but the clarity of many others has been seriously impaired by the process of reproduction. The argument supported by these photographs and the stylized explanatory drawings which are presented as alternative original evidence is not compelling. Once again valuable observations and sweeping, unproved assumptions are distressingly entangled. The chromatin bodies, for example, are described as being inside a nucleus, but no nuclear envelope is shown in any one of 46 illustrations. As far as one can tell from the photographs the chromatin bodies lie, as usual, directly in the cytoplasm. The author's figures are said to illustrate a sequence of nuclear changes in a series of comparable diploid cell types, but the middle of the sequence is taken up by seven successive illustrations of very much smaller cells of quite a different type. It is thus difficult to see in the alleged nuclear sequence anything but a heterogeneous collection of cell types arranged to illustrate a preconceived idea. The alleged process of meiosis is stated to give rise to tetrads of haploid cells, but four schematic drawings of cell pairs are shown with the two metaphase spindles of each pair in straight alignment. Such divisions, if consummated, would lead to four-membered cell *chains*. However, the coccus concerned does not form chains; the author's interpretation of the axis of division in these cells thus seems to be in error.

Further details are out of place in a general review. Here as elsewhere the study of the sequence of events in the living organism so promisingly initiated by Mason and Powelson (34) should soon lead to the resolution of present disagreements.

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[The history of bacterial caryology is not a history of discovery but mainly one of changing interpretations. Accordingly no attempt has been made to list all or even most of the papers on chromatin bodies known to the reviewer. Apart from references to matters of current controversy the papers cited are a representative sample of

publications which convey important facts about chromatin bodies in a manner which is intelligible to biologists in general. This has meant the exclusion of many valiant and devoted studies conducted according to private standards of significance and meaningful only to a small circle of bacteriologists. Fuller reviews of the subject will be found in articles by Piekarski (126) and DeLamater (73). The literature on bacterial nuclei from the beginnings of bacteriology to 1939 has been painstakingly assembled by Delaporte (1). A critical survey of bacterial cytology has recently been published by Winkler (127)].

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