OBSERVATIONS WITH THE ELECTRON MICROSCOPE ON THE FINE STRUCTURE OF THE NUCLEI OF TWO SPHERICAL BACTERIA

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ABSTRACT

The nuclei of two spherical bacteria have been examined in electron micrographs of thin sections of specimens prepared by the method of Ryter and Kellenberger (1958). The nuclei appear to consist of the same fine fibers in a matrix of low density which have already been seen in many other bacteria prepared by the same procedure. They are worth a separate description because their constituent fibers are arranged in patterns of uncommon orderliness. In the nuclei of one of the two bacteria this is seen at all times, in the nuclei of the other one only at the beginning of the growth cycle. In some places the diameter of the nuclear fibers is close to that of the DNA molecule in the model of Watson and Crick (1953).

INTRODUCTION

The appearance of bacterial nuclei (nucleoids, nucleoplasms) in the electron microscope varies with the manner in which they are prepared and may be either coarse (3) or fine grained (2). An interesting example of the latter type of nuclear structure—a meshwork of fibers which fills the nuclear region evenly and completely—is seen in bacteria that have been fixed and embedded for electron microscopy by the method of Ryter and Kellenberger (17). In most published micrographs the fibers of nuclei fixed according to this procedure appear to be arranged at random and form a feltwork. It seems worth while therefore to report observations on two bacteria in which the nuclear fibers are usually or at certain times arranged in patterns of a higher degree of orderliness. A few electron micrographs in which nuclei are visible in one of these organisms have already appeared in a different context elsewhere (5, 6).

MATERIALS

The nuclear structures to be described are those of two cocci, one relatively large and the other very small. The larger coccus, which will be referred to as "strain C" until it has been properly described and named bacteriologically, was first encountered four years ago as a contaminant on meat digest agar. It has been maintained in the laboratory because its nuclei are clearly visible during life, unusually easy to stain directly, and neatly brought out by the Feulgen procedure. On agar the coccus grows slowly and during the first 24 hours forms flat rectangular arrays consisting of one layer of cells. Crowding, so common after a few hours' growth in cultures of ordinary bacteria, is thus long delayed. Some features of the cell wall of strain C cocci have already been described elsewhere (12).

The other organism is a coccus so minute that it can only just be seen but not resolved internally with the light microscope. This bacterium, as discovered by A. C. Ruys, is a constant companion of certain strains of *Mycoplasma* (PPLO) isolated from man (5, 6, 16). The small coccus can be obtained pure by cultivating it on relatively simple media which do not support the growth of the *Mycoplasma* (16).

METHODS

The nuclei of strain C cocci have been examined with the phase contrast microscope by the method of

Mason and Powelson (9). For the Feulgen test cocci fixed for 1½ minutes with osmium tetroxide vapor were hydrolyzed for 9 minutes at 60°C. in N/1 HCl, rinsed with water, left for 3½ hours in Schiff reagent prepared with "Diamant Fuchsin" (Chroma Co., Stuttgart, Germany), rinsed with five changes of SO_2 water and for 20 minutes in running tap water, and then mounted over a drop of aceto carmine. This reagent prevents shrinkage, matches the refractility of the cocci better than water, and enhances the visibility of Feulgen positive structures (10, 14). Unhydrolyzed controls gave a negative reaction. Comparable but more brightly stained preparations of osmium-tetroxide–fixed C cocci were obtained with the azure A/SO_2 method of Huebschman (4). For electron microscopy both kinds of cocci were fixed by the method of Ryter and Kellenberger (17). The C cocci (from plate cultures) were embedded in vestopal W, the *Mycoplasma* cocci (from PPLO colonies in agar) in methacrylate. Details of the procedure followed in preparing the latter organisms for electron microscopy have been given in an earlier paper (6). Sections were cut on a "Sjöstrand microtome" and examined in a Philips electron microscope modified after designs of Le Poole and Kramer at Delft.

RESULTS

A. *Strain C Cocci*

In each living coccus phase contrast microscopy reveals one or two nuclei in the shape of slender curved bars, hooks, or crescents of relatively low density (Fig. 1). The nuclei give a distinct positive Feulgen reaction, best seen in aceto carmine (Fig. 2), but clearly positive also in water mounts. Time-lapse phase contrast micrography and comparison of the nuclear structures in fixed and strained cocci representing different stages of growth have shown that the basic shape of the nucleus of C cocci is a ring or crescent and that the nucleus divides into two rings by elongation and constriction (Fig. 3).

In electron micrographs of sections of cocci from 6 hour cultures the nuclei appear as thick bundles of more or less parallel fibers. These were often arranged at right angles to the plane of growth of developing transverse septa (Fig. 5). In most cases it was not possible to tell from the appearance of the randomly cut nuclei in which phase of their growth cycle they had been arrested by fixation (Fig. 6). However, several profiles have been found, such as those in Fig. 4, which by comparison with living cells and with stained preparations may reasonably be interpreted as sections of nuclei in division. Compact bundles of parallel fibers like those shown in Fig. 5 are most frequently seen in the nuclei of cocci from 6 hour cultures. The nuclei of cocci from 20 hour cultures are branched and their consitituent bundles of fibers follow tortuous courses (Figs. 7 and 8). At this stage the internal organization of the nuclei of strain C cocci is not very different from that of the nuclei of *E. coli* and *B. subtilis* as described by Ryter and Kellenberger (17).

B. *Mycoplasma Cocci*

These cocci can be made visible by staining (16) or by dispersing them in nigrosine but their nuclei are too small for resolution with the light microscope. However, in electron micrographs of sections a large part of the interior of *Mycoplasma* cocci is taken up by a mass of fibers (Figs. 9, 10–12) which by analogy with other bacteria can be regarded as the nucleus of these cells. During the fission of the cocci the central coil of fibers (coccus *A* in Fig. 11) is stretched and pulled

FIGURE 1

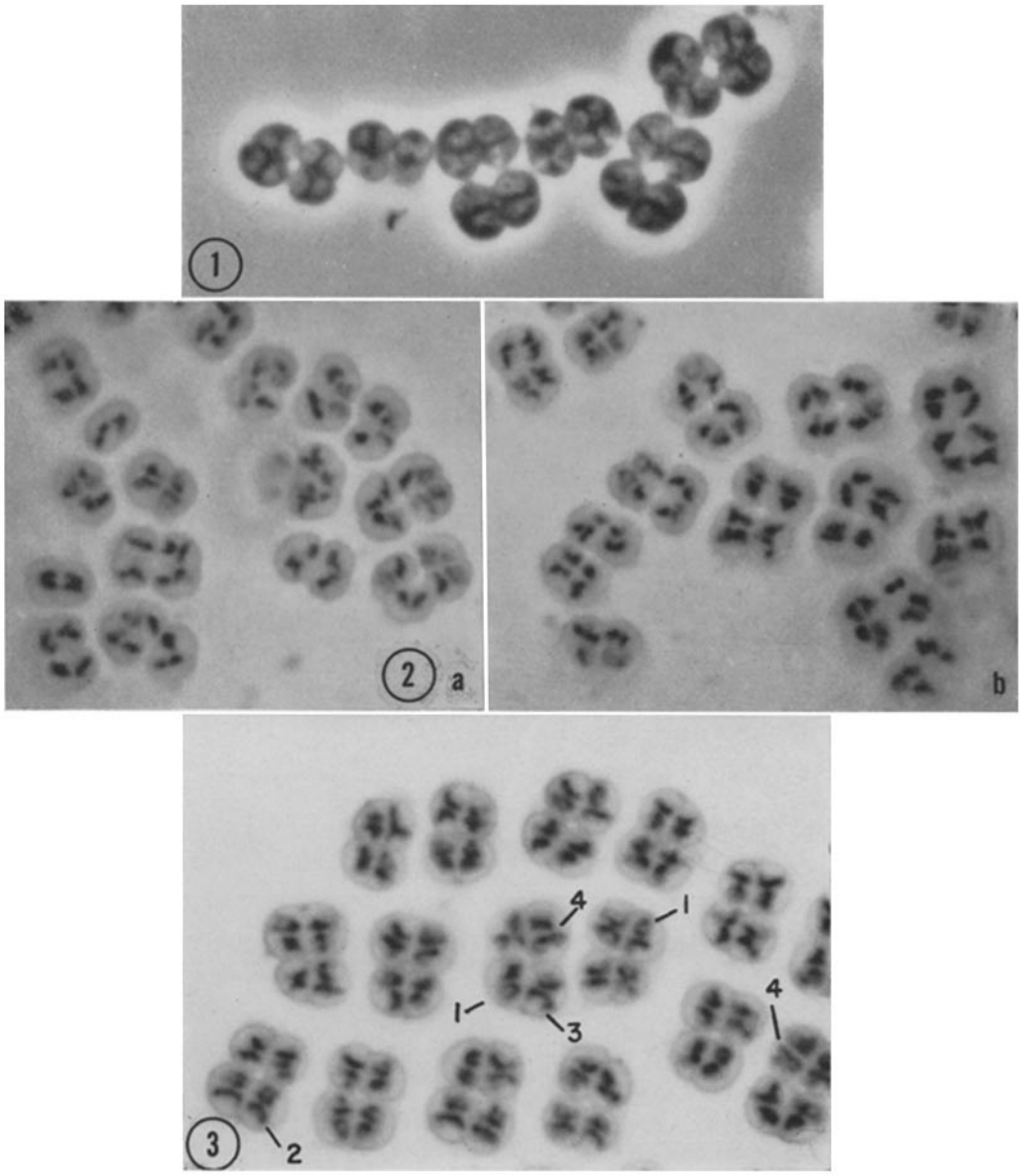
Phase contrast micrograph of C cocci that had been taken from a 6 hour plate culture and mounted in meat digest broth containing 20 per cent gelatin. The nuclei stand out white against the dark gray of the cytoplasm. × 2800.

FIGURE 2*a*, *b*

Feulgen reaction in C cocci from a 6 day old slant culture fixed *in situ* with osmium tetroxide vapor 6 hours after they had been spread on fresh meat digest agar. Photographed in aceto carmine. × 2700.

FIGURE 3

C cocci from an 18 hour culture on agar, fixed *in situ* with osmium tetroxide vapor. Stained with azure A-SO_2. Numbers 1 to 4 indicate successive stages in the duplication of the nuclei. × 3600.


1
2
a
b
4
1
1
3
4
3
2

out into two portions which for a time remain connected by a narrow bridge (Figs. 10 to 12). In reflecting so obviously a process of division, both in their shape and in the arrangement of their constituent fibers, the nuclei of *Mycoplasma* cocci differ from those of *E. coli* and many other bacteria in which there is usually no obvious correlation between the shape of the nuclear vacuole and the disposition of the crisscrossing fibers in its interior. Sections of nuclei of *Mycoplasma* cocci give the impression of being slices cut from a many-stranded coiled rope but serial sections are required to test the truth of this.

DISCUSSION

The very thin, contrasty nuclear fibers here illustrated are visible only in fixed and sectioned material but several points can be argued in favor of their existence in the living cocci. To begin with, it has several times been pointed out that the amount of deoxyribonucleic acid (DNA) which bacteria are known to contain can only be accommodated in their nucleus if the thread-like molecules of this polymer are tightly coiled or folded (1, 7, 15). Nucleoplasms consisting of coiled fibers are therefore just what one would expect to find in bacteria. By the same reasoning the diameter of the nuclear fibers ought to be of the order of size of the diameter of DNA molecules. The values actually found lie between 20 A (Fig. 9) and 60 A (Fig. 5). In view of the many uncertainties that attend their determination and the probable presence of precipitates on the fibers, caused by the fixative, these values do not appear too far removed from the 20 A which is the diameter of a double helix of DNA in the Watson-Crick model (18). In presenting this argument we have been following Kellenberger and his collaborators (7, 8, 17) who, having observed the high density of the DNA-filled heads of bacteriophages in sections of infected bacteria, regard the dense fibrous material in the nucleoplasms as DNA not combined with a protein partner. Similarly the 40 A fibers observed among others in spermatid nuclei are interpreted by Ris (13) as corresponding to the nucleoprotein which makes up these nuclei. A critical discussion of this point of view and of the alternative one, that the DNA is in the *low* density component of bacterial nuclei, has been presented by R. G. E. Murray (11). Fiber diameters of about 20 A repeatedly found in the present material seem to support the view (19) that in bacteria most of the length of the DNA molecule is not associated with proteins as in animals or plant cells.[1]

It is characteristic of the nuclei in electron micrographs of thin sections of bacteria fixed by the method recommended by the Geneva laboratory (17) that they have the same low density all through. When they are examined in the living cell with the phase contrast microscope bacterial nuclei also appear as objects of uniform and very low density. Even in Feulgen preparations there

[1] *Note added in proof:* A more detailed discussion of the fine structure of bacterial nuclei will be found in a recent paper on The finest structure of *Streptomyces coelicolor*. II. The nuclear material, by D. A. Hopwood and A. M. Glauert, 1960, *J. Biophysic. and Biochem. Cytol.*, **8**, 267.

FIGURE 4

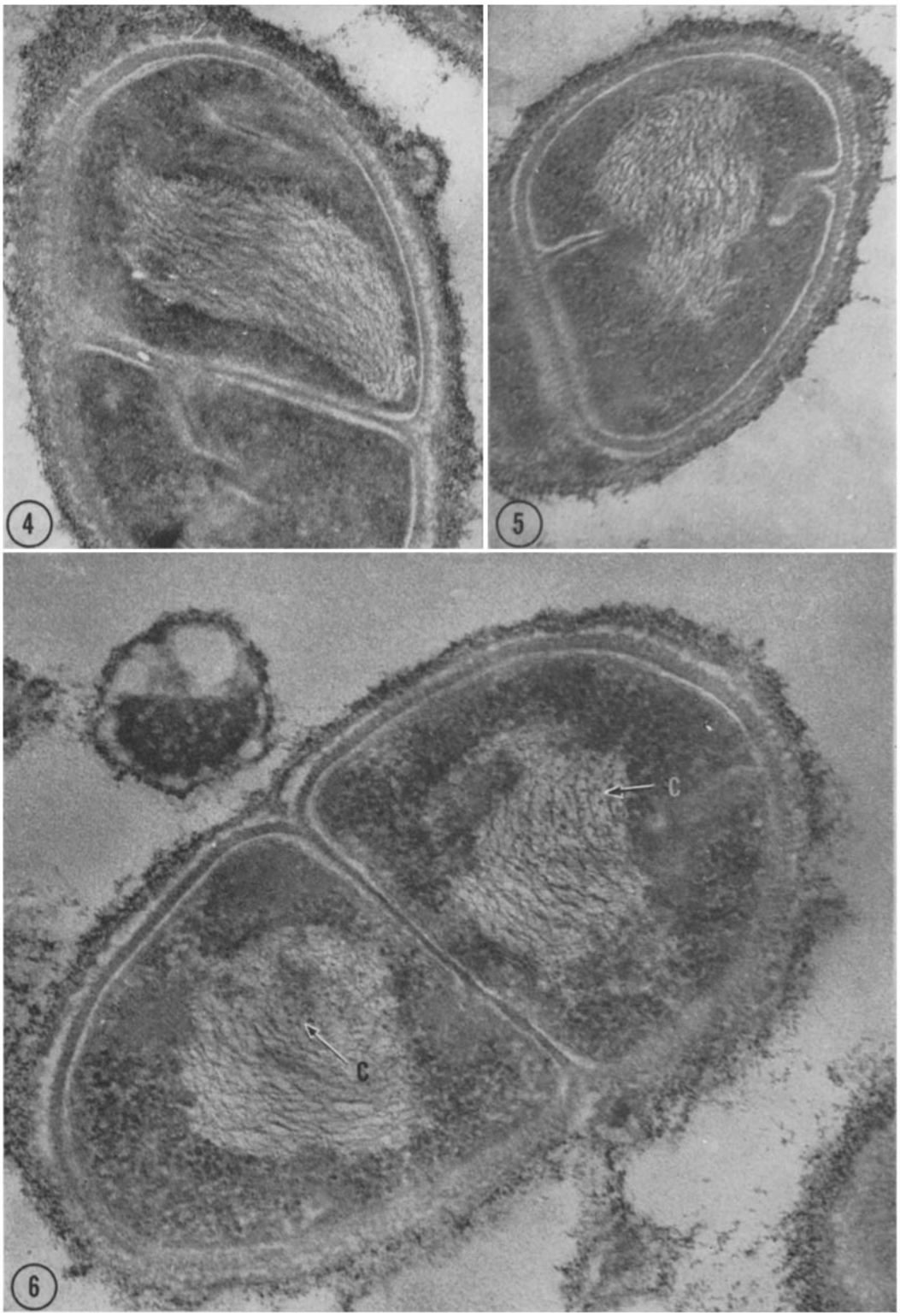
Electron micrograph of a section of a C coccus from a 6 hour culture on agar. The nuclear area is relatively large and probably in a state of division. × 72,000.

FIGURE 5

C coccus from 6 hour culture on agar. In this section the electron dense fibers are arranged more or less parallel to each other. Their main orientiation appears to be at right angles to the developing septum. × 70,000.

FIGURE 6

C coccus from 6 hour culture on agar. More highly enlarged than the micrographs of the two previous illustrations. Some of the fibers are transversely sectioned almost approaching cross-sectioning, *e.g.*, at *C* (*cf.* Fig. 7). × 114,000.


4
5
C
C
6

is a notable lack of graininess. It is therefore not unreasonable to assume that the nucleoplasms of the two cocci which are the subject of this communication are in fact during life filled with bundles of DNA-containing fibers. The mode of duplication of bacterial nuclei is still far from clear. For example, it is not known whether a nucleoplasm represents a single much coiled filament or several intertwined ones. An ingenious model of the bacterial nucleus which assumes the truth of the former alternative has recently been tentatively proposed by Kellenberger (7). The nuclei of the *Mycoplasma* cocci would seem to provide useful material for a test of the general validity of this model.

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REFERENCES

1. ANDERSON, T. F., *Cold Spring Harbor Symp. Quant. Biol.*, 1958, **23**, 47.
2. CHAPMAN, G. B., and HILLIER, J., *J. Bact.*, 1953, **66**, 362.
3. GIESBRECHT, P., and PIEKARSKI, G., *Arch. Mikrobiol.*, 1958, **31**, 68.
4. HUEBSCHMAN, G., *Mycologia*, 1952, **44**, 599.
5. ITERSON, W. VAN, and RUYS, A. C., *Antonie van Leeuwenhoek*, 1960, **26**, 9.
6. ITERSON, W. VAN, and RUYS, A. C., *J. Ultrastruct. Research.*, 1960, **3**, 282.
7. KELLENBERGER, E., The physical state of the bacterial nucleus, *in* Microbial Genetics, *10th Symp., Soc. Gen. Microbiol.*, Cambridge, 1960, 39.
8. KELLENBERGER, E., RYTER, A., and SÉCHAUD, J., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 671.
9. MASON, D. J., and POWELSON, D. M., *J. Bact.*, 1956, **71**, 474.
10. MCINTOSH, D. L., *Stain Techn.*, 1954, **29**, 29.
11. MURRAY, R. G. E., *in* The Bacteria, (I. C. Gunsalus and R. Y. Stanier, editors), New York, **1**, 1960.
12. MURRAY, R. G. E., and ROBINOW, C. F., Abstracts of communications, *7th Internat. Congr. Microbiol.*, (G. Tunevall, editor), Stockholm, Almqvist and Wiksell, 1958, 427.
13. RIS, H., Chemie der Genetik, *9th Colloq. Ges. Physiol. Chem.*, Berlin, Springer Verlag, 1959, 1.
14. ROBINOW, C. F., *Canad. J. Microbiol.*, 1957, **3**, 771 and 791.
15. ROWAN, J. W., and NORMAN, A., *Arch. Biochem. and Biophysics*, 1954, **51**, 524.
16. RUYS, A. C., *Antonie van Leeuwenhoek*, 1960, **26**, 1.
17. RYTER, A., and KELLENBERGER, E., *Z. Naturforsch.*, 1958, **13b**, 597.
18. WATSON, J. D., and CRICK, F. H. C., *Nature*, 1953, **171**, 964.
19. WILKINS, M. H. F. and ZUBAY, G., *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 55.

FIGURE 7

Section of C coccus from a 20 hour culture on agar. The nucleoplasms appear to be twisted in a tortuous manner. Longitudinal sections of fibers at *A*, cross-sections at *C*, in the latter diameters were found to range from about 20 to 60 A. The size and density of transversely sectioned fibrils will generally be greater than those of fibrils lying longitudinally or obliquely in the thickness of the section. This is so because the density in normal sections is given by a column of material equal in length to the thickness of the section; any "tilt" in transverse sections may increase the apparent diameter. × 100,000.

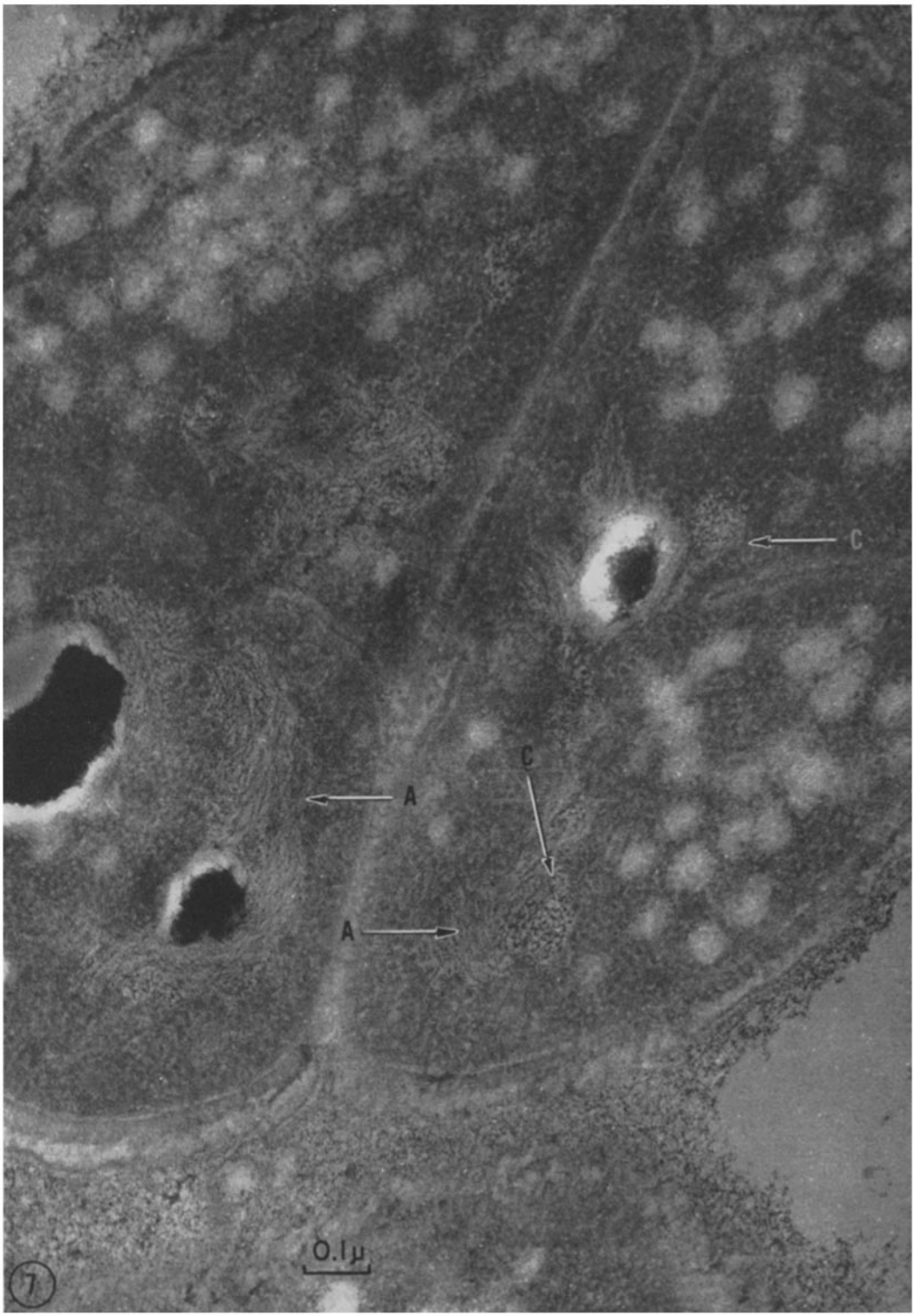

C
C
A
A
0.1μ
7

FIGURE 8

C coccus from a 20 hour culture on agar. The print of this micrograph has been reversed. Nuclear fibers stand out white against a dark matrix. Two very thin ones are indicated by arrows. × 140,000.

FIGURE 9

Of the two *Mycoplasma* cocci in this micrograph the upper one seems to have been cut equatorially and the lower one through its periphery. Fiber diameters close to 20 A. × 200,000.

FIGURE 10

Mycoplasma coccus in advanced stage of division. Printed in reverse to enhance the visibility of the fibers (white). × 180,000.

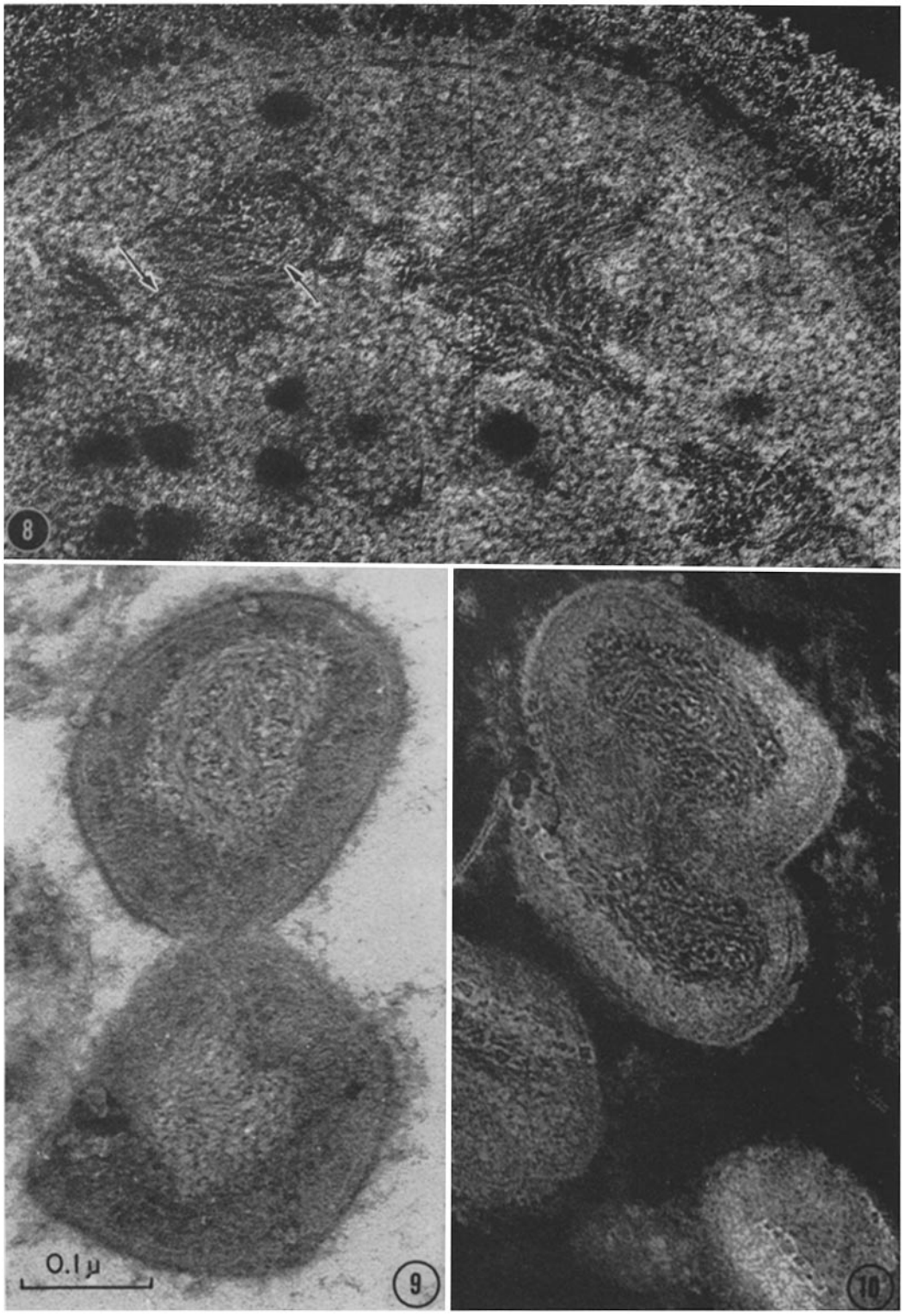
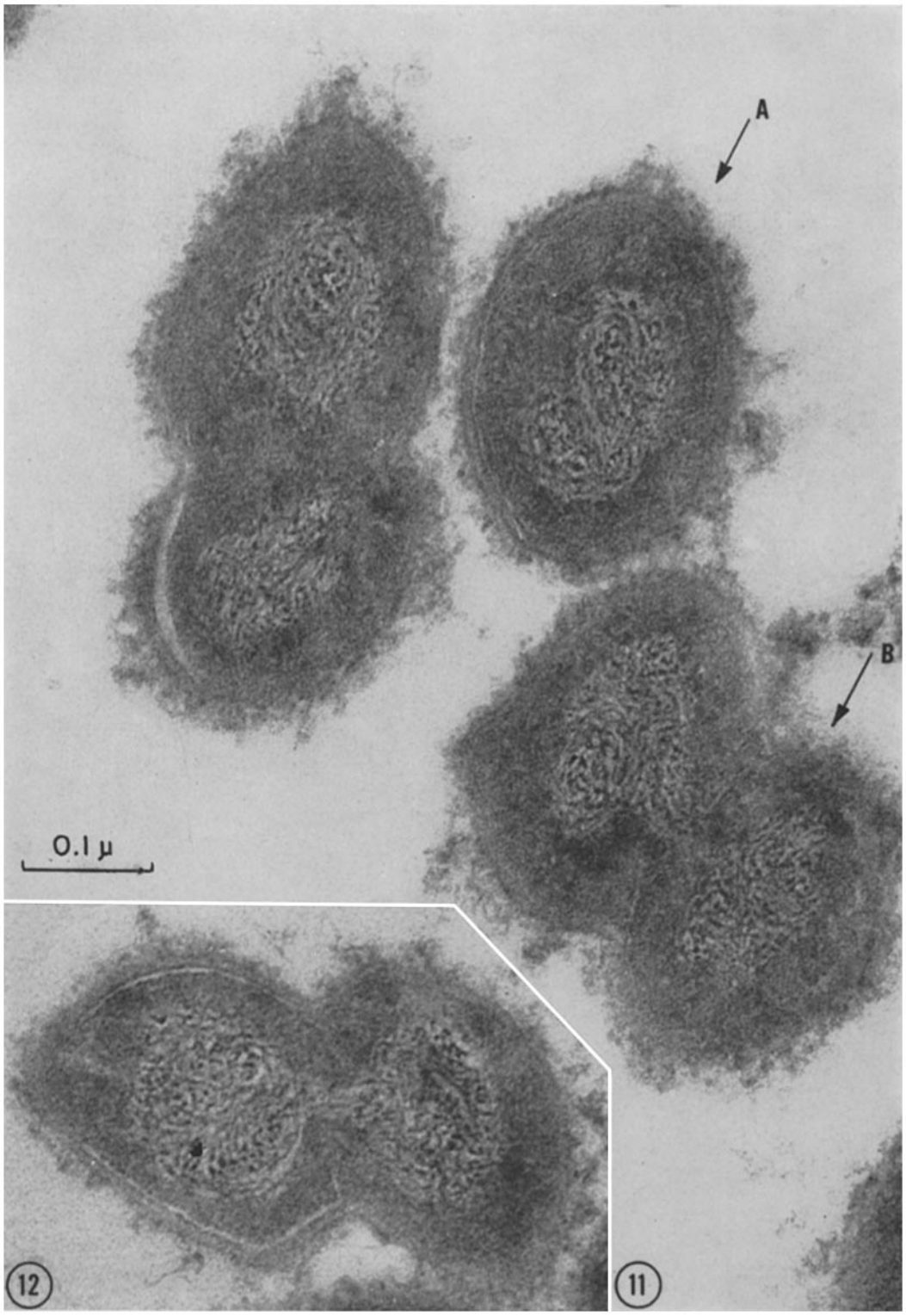

8
0.1 μ
9
10

FIGURE 11

Mycoplasma cocci. In coccus *A* the nucleus may be in an early phase of duplication. Division is almost completed in coccus *B* and the complicated fiber patterns of the daughter nuclei suggest that renewed duplication may already have begun. × 200,000.

FIGURE 12

Mycoplasma coccus in fission. The fibrous nucleoplasm has been pulled out into two portions still connected by a narrow bridge. × 200,000.


A
B
0.1 μ
12
11