DEMONSTRATION OF THE CHROMATINIC BODIES OF ESCHERICHIA COLI AND PROTEUS VULGARIS WITH THE AID OF THE PHASE CONTRAST MICROSCOPE[1]

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Chromatinic bodies (chromosomes, nucleoids, and nuclei) have been demonstrated in bacteria by various workers using a variety of methods (Peshkoff, 1938; Knaysi, 1942; Robinow, 1945; Knaysi and Baker, 1947; Duguid, 1948; Robinow and Cosslett, 1948; Hillier, Mudd, and Smith, 1949; Eisenstark and McMahon, 1949). Many of these methods involve the study of dead cells, and the stages in the division and distribution of the chromatinic bodies relative to the division of the bacteria have necessarily been reconstructed by logic. Some attempts to study this problem in living cells have been made by Piekarski (1939) and Peshkoff (1946).

The purpose of the present investigation is to demonstrate the chromatinic bodies in young, living bacteria and to determine the behavior of these bodies in actively growing and dividing cells of *Escherichia coli* with the aid of the dark phase contrast microscope.

METHODS

The organisms used in this investigation were from a laboratory stock culture of *Escherichia coli* and *Proteus vulgaris* OX-19 obtained from the United States Public Health Service.

An inoculum from an 18- to 24-hour-old brain heart infusion broth culture was spread over the surface of nutrient agar containing 5 per cent normal horse serum. After 1 hour at 30 C, two adjacent squares of agar were removed. On one, which was used to make an impression film, the bacteria were fixed on the agar block by exposure to the vapors of a 2 per cent solution of osmium tetroxide for 2 minutes (Robinow, 1942). An impression film was made on a slide, allowed to air-dry for 15 seconds, and then stored in 70 per cent alcohol. The films were hydrolyzed in N HCl at 60 C for 7 minutes, washed in tap water, and stained in a filtered 0.2 per cent aqueous solution of basic fuchsin. The second portion of agar was mounted between two sterile cover glasses, sealed with sterile "vaspar," and examined with the phase contrast microscope. In this way a comparison of living and fixed cells of the same age could be made. A similar preparation was used to observe and photograph the behavior of chromatinic bodies in growing and dividing bacteria. In this case a single cell or group of cells was chosen and followed until a microcolony developed and cell division no longer took place. The preparation was incubated at room temperature.

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A Spencer 95× oil-immersion dark M phase contrast objective was used in conjunction with a 10× eyepiece and a Wratten E (orange) filter for the examination of living cells. The stained films were examined in a bright field using a 95× oil immersion achromatic objective together with a 10× eyepiece and Wratten G (yellow) and H (blue) filters. A Bausch and Lomb ribbon filament research lamp was used in both cases. Photographs were taken on Kodak M plates at a magnification of 1,140 diameters.

Microchemical tests for fat, glycogen, and volutin were performed. Sudan black B (Hartman, 1940) was used for the detection of fat. For the determination of

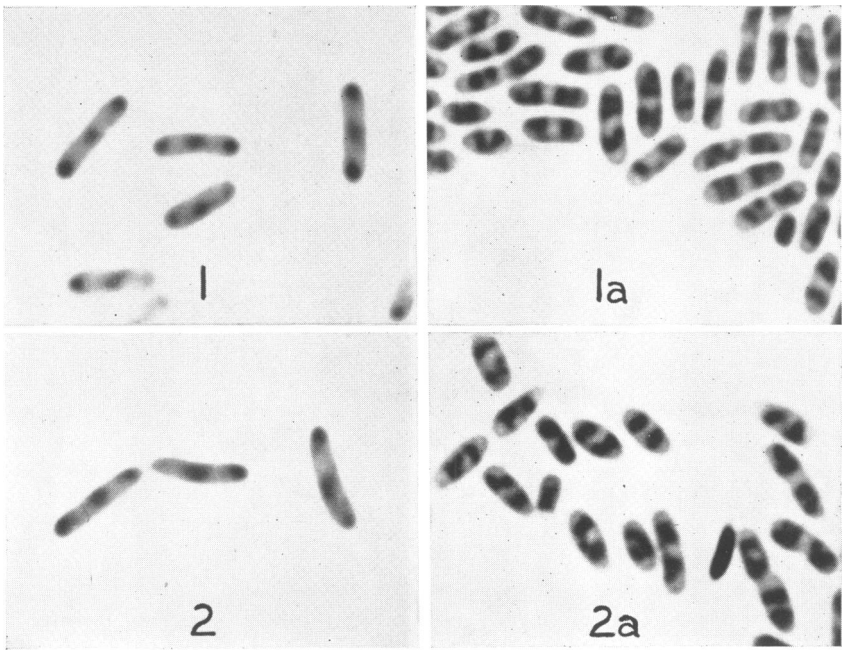


Figure 1. One-hour-old living cells of *E. coli* photographed with the dark phase contrast microscope; × 3,700.

Figure 1a. One-hour-old cells of *E. coli* fixed, hydrolyzed, and stained. Photographed with the bright-field microscope; × 3,700.

Figure 2. Proteus vulgaris OX-19. Treatment as in figure 1.

Figure 2a. Proteus vulgaris OX-19. Treatment as in figure 1*a*.

glycogen, the bacteria were first suspended in 70 per cent alcohol for 5 to 10 minutes and then transferred to Lugol's iodine solution. To determine the presence of volutin, the bacteria were suspended in dilute (0.02 and 0.0002 per cent) aqueous methylene blue solution.

RESULTS

Young cells of *E. coli* (figure 1) and *P. vulgaris* (figure 2) when examined with the dark phase contrast microscope are found to be differentiated into light and dark areas. A comparison with fixed, hydrolyzed, and stained cells (figures 1*a*

and 2*a*) from the same cultures shows that the light areas in the living cells occupy the same position as the darkly staining chromatinic bodies. The dark areas correspond in position to the faintly staining cytoplasmic portions of the fixed cells. In the living cells the light areas will henceforth be referred to as chromatinic bodies and the dark areas as cytoplasm. With the bright phase contrast microscope, the picture is reversed. The chromatinic bodies appear dark as compared with the lighter cytoplasm.

The division and distribution of the chromatinic bodies in growing and dividing cells can be followed with the dark phase contrast microscope. The following description is for *E. coli*. Shortly after the transfer of the bacteria from broth to agar, the differentiation into light and dark areas within the cell is apparent; it becomes more distinct after about 1 hour's incubation at room temperature. A few cells contain only one chromatinic body but most contain two.

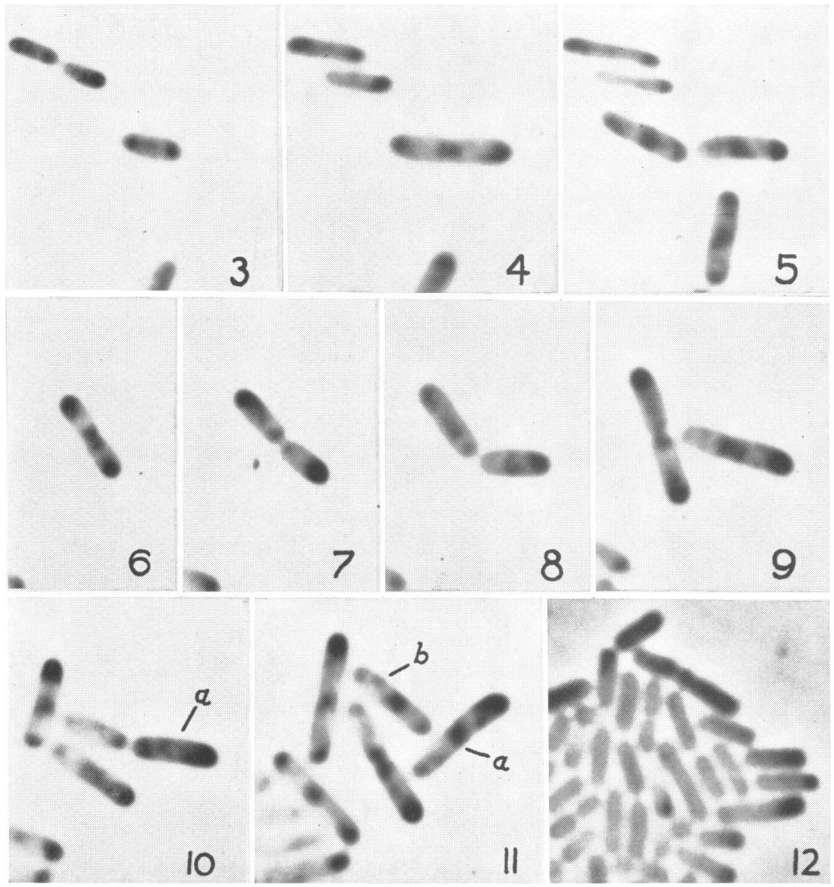
When only a single chromatinic body is present (figure 3), cell division is preceded by the division of this body into two. A dark, transverse band separates each half (figure 4). Cell division is first evidenced by the appearance of an indentation on either side of the cell, and the cytoplasm is soon afterward split by a narrow light space. There results a symmetrical distribution of one chromatinic body to each of the sister cells. When a pair of chromatinic bodies is already present in the parent cell, the cell usually divides as above (figure 6, 7, and 8). Occasionally, however, each chromatinic body may divide, giving rise to a cell containing four chromatinic bodies. Cell division then results in two cells each containing a pair of the bodies. At least two chromatinic bodies are present in each cell before it divides.

The details in the division of the chromatinic bodies are difficult to observe. Usually the body becomes somewhat darker and less distinct after cell division. A short time later there appears a pair of light areas with a darker band separating them (cell *a* in figures 10 and 11). In some cases, division is preceded by the appearance of a small, dark patch to one side of the chromatinic body (cells *a* and *b* in figure 11). In all cases the chromatinic bodies appear to divide transversely to the long axis of the cell.

The differentiation into chromatinic bodies and cytoplasm is easily observed during the late lag and logarithmic periods of growth. As cell divisions become less frequent and the cells become smaller, the cytoplasm appears paler until finally the majority of the cells assume a homogeneous appearance (figure 12).

In an attempt to determine why young cells show an internal differentiation with phase contrast microscopy, tests for fat, volutin, and glycogen were performed. None of the cells give a positive reaction for fat when treated with Sudan black B. With the 0.02 per cent methylene blue solution, the majority of the cells from the broth culture stain uniformly pale blue. After about $\frac{1}{2}$ hour's incubation on the agar surface the bacteria stain more heavily. The portions corresponding to the cytoplasm stain dark blue, whereas the portions corresponding to the chromatinic bodies stain lighter blue, which has a tinge of red purple. This pattern is not altered after similar cells are treated at 80 C for 5 minutes. After 1 to $1\frac{1}{2}$ hours' incubation on the agar surface, the bacteria stain almost

uniformly dark blue. Hot water treatment of similar cells removes the basophilic material masking the chromatinic bodies. The cells now appear like those incubated for $\frac{1}{2}$ hour. It is not known whether this extractable basophilic material is present in the chromatinic bodies or merely forms a surrounding layer.



Figures 3 to 5. Division of a cell of *E. coli* containing a single chromatinic body. Photographed with the dark phase contrast microscope; × 3,700. Age of culture is 1, $1\frac{1}{2}$, and 2 hours.

Figures 6 to 12. Behavior of chromatinic bodies during the formation of a microcolony by *E. coli*; × 3,700. Age of culture is 1, $1\frac{1}{4}$, $1\frac{1}{2}$, 2, $2\frac{1}{4}$, $2\frac{1}{2}$, and $5\frac{1}{4}$ hours.

With the 0.002 per cent methylene blue solution, a similar but much fainter differential staining of the cell interior is obtained with many of the young cells. The cytoplasmic regions stain darker blue after treatment at 80 C for 5 minutes.

Staining with Lugol's iodine fails to reveal the presence of glycogen in either organism from the broth cultures or in young cells of *Proteus* grown on agar. The cytoplasmic portions of young cells of *E. coli* stain gray brown, which is not characteristic of glycogen. The results of the microchemical tests indicate the absence of fat and the possible absence of volutin and glycogen.

DISCUSSION

With dark phase contrast, those objects having a greater optical path (thickness × refractive index) appear darker than others of smaller optical path (Richards, 1946). Since the chromatinic bodies are as wide as the cell, they are probably as thick as the cell. The dark and light banding of the cells, therefore, is probably largely due to a difference between the refractive indices of the cytoplasm and chromatinic bodies.

Since bright phase contrast renders objects of greater optical path brighter than those of smaller optical path, the chromatinic bodies would be expected to appear darker than the cytoplasm. This has been confirmed by observation.

Eisenstark and McMahon (1949) and Eisenstark, McMahon, and Eisenstark (1950) compared fixed, hydrolyzed, and stained cells of *Azotobacter agile* with similar cells that were fixed and observed by phase contrast microscopy. These methods revealed light and dark transverse bands in the young cells. Since the type of phase equipment used was not mentioned, it is difficult to evaluate these results with those of the present investigation.

Studies with the electron microscope by Robinow and Cosslett (1948) on *Erwinia carotovora*, *Pseudomonas* sp., *Corynebacterium* sp., and gram-positive spore-forming bacteria and by Hillier, Mudd, and Smith (1949) on *E. coli* have shown the chromatinic bodies to appear lighter than the denser cytoplasm. Although the general appearance of these bacteria agrees with that seen by the use of dark phase contrast, it is at present uncertain how they are to be correlated, since image formation by the two instruments does not necessarily depend upon the same factors.

Peshkoff (1938) has demonstrated chromatinic bodies in living *Achromobacter epsteinii* from a 24-hour-old culture by the use of bright-field microscopy. The chromatinic bodies appear light in contrast to the darker cytoplasm. The bodies were believed to be demonstrable because they are more refractile than the cytoplasm. The results of the present investigation, however, indicate that when a difference in refractive index exists in the case of *E. coli* and *Proteus* it is the cytoplasm that is the more refractile.

Young cells of *Serratia marcescens* growing in a slide culture showed a differentiation into light and dark portions (Piekarski, 1939) corresponding to the pattern shown in the present investigation by dark phase contrast microscopy. Piekarski believed that the dark bodies corresponded to the Feulgen-positive bodies in fixed cells of the same age. He also found, however, that these dark bodies did not always correspond in position to bodies that absorb ultraviolet light. In view of the results of the present investigation, it is more likely that the light portions represented the Feulgen-positive (chromatinic) bodies and the dark portions represented cytoplasm.

Chromatinic bodies in living cells of *E. coli* never assume the appearance of dumbbell forms, which have been described by Robinow (1942, 1944) and which have been seen in the fixed, hydrolyzed, and stained cells in this investigation. The dumbbell forms may be either artifacts resulting from contraction during

fixation or structures revealed only after hydrolysis has removed portions of the chromatinic bodies as they appear in living cells.

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ADDENDUM

After this paper had been submitted for publication the October, 1949, issue of the *Comptes rendus des séances de la société de biologie* was received by the University of Pennsylvania library. This journal contains two papers by R. Tulasne, who reports the demonstration of light chromatinic bodies against a darker cytoplasm in living *Proteus vulgaris* and *Escherichia coli* by the use of the phase contrast microscope. The stages in the division of the bodies are illustrated by drawings. During the lag phase the chromatinic bodies appear as rounded forms, but during the logarithmic phase the bodies are rodlike and assume U and V shapes during division. Thus the shapes of the chromatinic bodies in the living cells correspond closely to those in fixed and stained films. This was not always found to be the case in the present investigation.

SUMMARY

Dark phase contrast microscopy reveals the chromatinic bodies of young, living cells of *Escherichia coli* and *Proteus vulgaris* as light areas distinguished from the darker cytoplasm.

These young cells do not contain detectable fat. The test for glycogen is negative in *Proteus vulgaris* and inconclusive in *Escherichia coli*. The presence of volutin is doubtful.

Cell division does not occur until there are at least two chromatinic bodies present in the cell. One chromatinic body is distributed to each of the sister cells. The succeeding cell division is preceded by a division of the chromatinic body into two. The details in the division of the chromatinic bodies are difficult to observe.

Differentiation between chromatinic bodies and cytoplasm is distinct in the late lag and logarithmic phases. In later stages this differentiation is lost and the cells appear homogeneous.

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