Electron Microscope Study of DNA-Containing Plasms

II. Vegetative and Mature Phage DNA as Compared with Normal Bacterial Nucleoids in Different Physiological States*

BY EDOUARD KELLENBERGER, DR. ÈS SC., ANTOINETTE RYTER, LIC. ÈS SC., AND JANINE SÉCHAUD, LIC. ÈS SC.

(From the Laboratoire de Biophysique, University of Geneva, Geneva, Switzerland)

PLATES 330 TO 334

(Received for publication, July 21, 1958)

ABSTRACT

The nucleoids of *Escherichia coli*, independently of the physiological state of the bacteria, are shown to be preserved as a fine-stranded fibrillar nucleoplasm by an OsO_4 fixation under defined conditions: acetate-veronal buffer pH 6, presence of Ca^{++} and amino acids, stabilization with uranyl-acetate before dehydration. The same fixation procedure applied to the DNA of vegetative phage reveals a pool of homogeneous fibrillar structure very similar to the nucleoplasm. The "versene test," which produces a coarse coagulation of these plasms, emphasizes the similar behaviour of the pool and the nucleoids.

The heads of mature phage are preserved in their true polyhedral shape by the standard fixation procedure, although they may be badly distorted when fixed under different conditions. Lanthanum nitrate and uranyl-acetate are shown to increase markedly the contrast of both phage and cytoplasm.

The consequences of the fibrillar structure of the genetic material are discussed in relation to the probable division process.

INTRODUCTION

A systematic study of the response of bacterial nuclei to fixation has resulted in the establishment of standard fixation conditions which produce a fine-stranded nucleoplasm in the growing cells of the 5 bacterial species observed (38). The same fine-stranded nucleoplasm is also observed when the cells are in various other physiological states. We report here our observations on resting cells obtained by starvation or by treatment with chloramphenicol.

The fine structure of the nucleoids in all cases considered led one to ask whether other plasms also composed mainly of desoxyribonucleic acids would present the same structure when fixed under the standard conditions. For this comparative study, we chose the DNA of vegetative and mature phage T2. T2 was used because, upon infection, there is a breakdown of the bacterial nucleus which ensures that further observations will not be simply of the persisting nucleoid. Mature phage was observed intracellularly, because the manipulations of free phage during fixation were found to be difficult.

The comparative fixation behaviour of these different DNA-containing plasms was also studied by the application of a versene treatment of previously fixed bacteria which produces a coarse appearance of the bacterial nucleoplasm.

In an infection of *E. coli* by phage T2, the breakdown of the bacterial nucleus is accompanied by formation of marginal vacuoles containing DNA (6, 30, 32–34), from which the pool of phage DNA develops some minutes later (27). This pool had already been predicted from genetic studies on the development of phage (41), and could be morphologically observed in single cell bursts (22). The time of appearance of the pool as cytologically observed agrees with chemical studies, which show that the phage DNA starts to increase 6 to 7 minutes after infection (7, 16). Then about 9

* This work was supported by the "Fonds National Suisse pour la Recherche scientifique."

minutes after infection, protein precursors, immature phages, and finally, at 13 minutes, complete phages appear in the infected cell (see reference 26).

If chloramphenicol, which is a specific inhibitor for protein synthesis (43), is added 8 minutes after infection, but before phage proteins appear, the synthesis of proteins is completely inhibited but the synthesis of phage DNA is not affected (17, 40). By means of this drug it was possible to build up a huge DNA pool in the absence of bacteriophage completion. It has been shown that phage DNA formed in the presence of chloramphenicol is normal, since it is used in building up phages as soon as the inhibitor is removed (17). In the present paper we will describe and discuss the fixation behaviour of this giant pool of vegetative phage-DNA. We will see that it must have a structure very comparable to the nucleoplasm of bacterial nucleoids.

Mature, infectious phages have a head composed of a thin protein membrane enclosing DNA probably associated with a polyamine (2). The morphology of phage T2 has been well studied by at least five fundamentally different preparation methods which showed its head to be polyhedral (3, 15, 18, 21, 42). There can be, therefore, no doubt about its shape, and ultrathin sections must give consistent results. In earlier observations intracellular bacteriophage appeared very distorted (32). In this paper we give improved results on intracellular phage, owing to our improved fixation methods.

We deliberately restrict ourselves here to the study of these two phage-DNA plasms and comparison of them with the bacterial nucleoplasm. The more general problem of intracellular phage formation and maturation will be treated in another paper (27).

Material and Methods

Bacterial Strains.—*E. coli* B, provided by the Virus Laboratory, Berkeley, and K12 S.

Bacteriophage.—T2 (26). For general phage techniques see Adams (1).

Growth Medium.—Bacto-tryptone 1 per cent, 0.5 per cent NaCl, pH 7–7.2.

Chloramphenicol Solution.—A stock solution of chloramphenicol[1] was made in distilled water and stored in the cold for not longer than 2 weeks.

Solutions for Fixation and Embedding:

1. Acetate-veronal buffer of Michaelis: basic mixture: 19.428 gm. Na-acetate, 29.428 gm. Na-veronal, 34 gm. NaCl, distilled water to 1000 ml. Mix 5.0 ml. of the basic mixture, 7.0 ml. HCl N/10, 13.0 ml. distilled water, and 0.25 ml. $CaCl_2$ M. The pH of this buffer is 6.1. It is used for the preparation of fixative, washing fluid, and the 2 per cent agar solution.

2. Fixative: 1 per cent OsO_4 dissolved in the acetate-veronal buffer.

3. Washing fluid: uranyl-acetate dissolved at 0.5 per cent in the acetate-veronal buffer; the pH goes down to about 5. In some experiments, a 1 per cent solution of lanthanum nitrate in acetate-veronal buffer was used.

4. Versene solution: versene (disodium ethylenediamine tetraacetate) 0.25 M in the acetate-veronal buffer.

5. Polyester embedding medium (25): vestopal W, purchased from M. Jaeger, Vésenaz, Genève.

Standard Fixation.—30 ml. of culture are mixed with 3 ml. of fixative and centrifuged at once for 5 minutes at 4000 R.P.M. The pellet is resuspended in 1 ml. of fixative and 0.1 ml. of tryptone medium and left during the night at room temperature. This suspension is diluted into 8 ml. of acetate veronal buffer, and centrifuged 5 minutes at 4000 R.P.M. The pellet is resuspended in about 0.03 ml. of 2 per cent agar, mixed well, and put as a drop on a microscope slide. These manipulations with the agar must be made at 45°. After cooling and gelation, the drop is cut into little cubes, which are treated 2 hours in the uranyl-acetate solution.

The Versene Test.—Instead of the treatment in uranyl-acetate, the blocks are treated 2 hours in the versene solution. This test is called positive if the DNA plasm becomes coarse, and negative if no significant changes are observed compared with the control treated in uranyl-acetate.

Embedding.—The blocks are dehydrated in acetone (25, 50, and 75 per cent during 15 minutes, 90 and 100 per cent during 30 minutes), and embedded in vestopal W by 30 minute immersions in 4 different vestopal mixtures (mixture I: 1 part vestopal—3 parts acetone; mixture II: 1 part vestopal—1 part acetone; mixture III: 3 parts vestopal—1 part acetone; mixture IV: vestopal + 1 per cent benzoyl peroxyde + 0.5 per cent cobalt naphthenate). The blocks are finally placed in gelatin capsules filled with mixture IV. The polymerization is made at 60° during 12 to 24 hours. For a more detailed description of the fixation and embedding see reference 38.

Microtome of our own design (20).

Electron Microscope.—RCA EMU 2D fitted with canalco platinum apertures in the condenser (10 mil) and in the objective (2 mil), and a canalco pole-piece compensator.

RESULTS

1. The Nucleoplasm of Bacterial Nucleoids in Different Physiological States:

(*a*) *Growing Cells.*—We have shown (38) the existence of a fibrillar nucleoplasm in all cells of a

[1] Gift from Parke, Davis & Co., Detroit.

growing *Escherichia coli* population fixed under the standard conditions (Figs. 1 *a* and *b*). The individual fibrils are very near the limits of resolution and contrast of our electron microscope, but even so, diameters of 30 to 60 A can be measured. Some of the nucleoid regions can be seen to show a parallel arrangement of the fibrils, which frequently seem to be associated by pairs. We have already pointed out that this double structure can be the image of the impregnation which takes the place of "fixation" in the case of DNA-plasms (38). But most frequently the nucleoplasm shows a sort of network with random distribution of component strands. We cannot yet decide to what extent this difference in arrangement of the fine structure is due to fixation, section thickness, or electron optical effects.

When conditions different from the standard ones prevail during processing, a more or less coarse coagulation of the nucleoplasm is produced. In particular, if our versene test (see Methods) is applied, the aspect changes in a very characteristic way (Fig. 1 *c*).

(*b*) *Nucleoids of Starved Bacteria.*—Growing cells are sedimented by centrifugation and resuspended in a 0.5 per cent NaCl solution of the same pH as the growth medium. During further incubation and aeration for 1 hour, the cells cease to grow, having used up their reserves. They are, however, still able to resume growth and to form colonies. By this method, resting cells are produced under more precise conditions than in an old culture. The nuclei of these cells become elongated and occupy the axial parts of the bacteria (19, 23). The nucleoplasm of such cells is again fibrillar (Fig. 2). No fundamental difference can be seen between this nucleoplasm and that of growing cells; the regions with parallel arrangement of the fibrils are visible only exceptionally, as already discussed for growing cells. Again the nucleoplasm responds to the versene test by coagulating.

(*c*) *Chloramphenicol-Treated Cells.*—When cells are treated with chloramphenicol or aureomycin, the synthesis of proteins is inhibited (11, 43). The synthesis of DNA, however, continues (13, 43). It has already been shown that the nucleoids of treated cells undergo a transformation leading to specific vesicular structure (19, 23, 24). This transformation is also obtained with sublethal doses of the drug, doses which allow even some residual cell division (19). It has been proposed (37) that this nuclear transformation is due to a disturbance of the permeability of the cell which allows chlorine ions to penetrate thus creating a salt effect. Our experiments with salt-free tryptone as growth medium show clearly, however, that the transformation is not a pure salt effect, since the same specific vesicular nucleoids are obtained. The fine structure of chloramphenicol-treated cells is again fibrillar (Fig. 3 *a*), exactly as already described for aureomycin (24). Here also the versene test is positive (Fig. 3 *b*).

2. The DNA Pool of Vegetative T2:

Exponentially growing cells ($2 \cdot 10^8$/ml.) of *E. coli* B are infected with a multiplicity of about 3 T2 phages per bacterium. Eight minutes later, 25 γ/ml. of chloramphenicol are added to the culture and incubation is continued. At different intervals the bacteria are fixed and embedded. Fig. 4 *a* shows such a cell fixed 75 minutes after infection. The DNA pool fills most of the cell. Its limiting interface with the cytoplasm is "rough"; many protrusions of cytoplasm penetrate the pool and are frequently cut as transverse sections, giving the impression of islets. The DNA-plasm of the pool is fine-stranded (Fig. 4 *b*); regions with parallel arrangement can be seen. Its response to the versene test is again positive (Fig. 4 *c*). The pool, therefore, behaves very similarly, if not identically, to the nucleoplasm of the bacterial nucleoid. From the observation of many sections we have the impression that the pool has a somewhat higher fibril concentration than the nucleoplasm. The pool obtained in the presence of chloramphenicol does not show any significant differences from the normal pool except in size (27). It may be interesting to note that chloramphenicol does not produce a vesicular arrangement of the DNA-plasm in the case of infected bacteria, as it does in the uninfected cells. The form and arrangement of the pool resemble very much the nucleoids of growing cells obtained in the absence of NaCl (38).

3. Intracellular Phage:

In order to obtain as high a number of intracellular phage as possible, we used lysis-inhibited cells. Growing cells of *E. coli* B at a concentration of $2 \cdot 10^8$/ml. were infected with a multiplicity of 3–5 T2 per bacterium. After 7 minutes they were superinfected with the same T2 phage in order to produce lysis inhibition (10). Thirty minutes after infection, the cells were fixed and embedded. When the standard fixation is used, the phage heads are polyhedral (Fig. 5). Deviations from the standard fixation lead to very deformed heads which have

no similarity to their known shape. The versene test, however, gives no very great deformations. The sizes of the heads are found to be 525 A x 710 A, which can be compared with 650 x 900 A in frozen-dried specimens (42) and 850 x 980 A in phage prepared by the agar filtration method (21). This would indicate that at least 20 per cent contraction in the linear dimensions occurs during dehydration and embedding. This value could be even higher, because in freeze-drying the existence of some contraction cannot be excluded *a priori*. Some contraction is probably general in polyester embedding; for bacteria, it is always manifested by a slight wrinkling of the cell wall. In standard fixation phage is only surrounded by a very small clear halo, while a very extended halo appears in "bad" fixations.

For intracellular phage, the tails cannot be seen except very rarely and with a great effort of imagination: the standard fixation does not provide enough contrast to reveal them. The membrane of the phage head, however, can be seen when the phages are cut in a suitable orientation.

4. The Increase of Contrast due to Lanthanum or Uranyl:

The standard fixation clearly results in very highly contrasted phages. We were interested to know if uranyl-acetate or lanthanum are responsible for this and if the increase of contrast is specific for the DNA of phage. In order to test this, we made a fixation without uranyl-acetate but maintaining carefully a sufficient concentration of Ca ions during the procedure (38) (Fig. 5 *a*). Other aliquots were treated with lanthanum nitrate (Fig. 5 *b*) or uranyl-acetate (Fig. 5 *c*). Both adjunctions increase the contrast, the most active being undoubtedly the uranyl-acetate. That this increase of contrast is not specific for DNA can be seen on Fig. 5 *d*, which is different from Fig. 5 *a* only by a photographically increased contrast. At least the cytoplasmic granules (ribonucleoprotein) are stained in addition to the phage.

It is interesting to note the different mean electron scattering in the nucleoplasm of the pool as compared to mature phage, both of which contain the same DNA. It is a clear demonstration of the influence of concentration: while phage contains less than 20 per cent water, the bacterial nucleus and the pool contain probably more than 80 per cent. Hence, the concentration in phage, and therefore the mean electron scattering, is about 5 times higher. This situation also explains why the increase of contrast due to uranyl-acetate cannot be observed for the pool or the nucleoids.

DISCUSSION

We have already discussed why the fine structure of the nucleoplasm is to be considered as preserved in a significant way by the standard fixation (38). We recall that this fixation alone gives reproducible results and is able: (*a*) to maintain polyhedral phage heads, (*b*) to produce fine nucleoplasm in the 5 bacterial species considered, and (*c*) to produce a fine-stranded pool of phage DNA.

If we accept as valid the results of this fixation, we may consider the inferences of our observations. Both the phage-DNA pool and the nucleoplasm of the nucleoids are composed of fibrillar material. The two different aspects of this material, a parallel arrangement or an irregular network-like appearance, could either be the reflection of two different physiological states, or represent the same arrangement, which would have to be regular, and differ only because of optical or geometrical phenomena. It could even be that only the parallel disposition of the fibrils is significant, the other being a slight coagulation artifact. For the present, nothing enables us to decide between these possibilities. More work has to be done to be able to interpret the very fine structure in a non-speculative way. It is likely that the improvements in electron microscopy and "staining" will help further.

Comparison between the nuclear structures of bacteria and those of higher cells is hazardous as long as systematic studies have not been made for the latter also. It is noteworthy, however, that Grell and Wohlfarth-Bottermann (12) found a fibrillar structure in the chromosomes of a Dinoflagellate, and De Robertis (9) in the chromosomes of a higher cell; both types contain fibrils with a width of 40 to 80 A, very comparable to those found in bacteria. De Robertis also found the presence of Ca ions to be important during fixation. These authors' observations should be compared and interpreted together with those of Ris (36), who concludes that the chromatin units are fibrils of 250 or 500 A in width.

Differences in structure can be expected, however, because of different chemical compositions. It is well known that the DNA of the nuclei of higher cells is associated with histone. Sometimes histone is replaced by a protamine or related compound. In bacteria and phage, however, an even

simpler polyamine seems to neutralize the free acid groups of DNA: the presence of spermidine and putrescine has been shown for *E. coli* and phage T4 (2). In considering response to OsO_4, pure nucleic acids do not react, while histone does (5); basic proteins of the protamine type are not reactive; the behaviour of the polyamines is variable: the hydrochlorides of putrescine and spermine do not react while the phosphates of spermine and spermidine do, though in alkaline media all four are slowly reactive (unpublished results). Hence it is very likely that different chromatins will behave very differently during fixation. In preliminary experiments with higher protista the versene test seemed to be negative (14). It may well be that this test will provide a tool able to show a difference between DNA-containing plasms of different chemical composition. Systematic studies along this line are being undertaken in our laboratory.

Replication of phage DNA is very rapid: every fraction of a minute a new phage genome is produced. On an average each genome performs 5 matings before incorporation into mature phage, all within a period of only some 8 minutes (8, 41). Hence arose the idea of the pool of phage DNA. The morphological picture of a localization of the DNA of the pool corroborates the genetic inferences and is therefore an indirect proof for the theories based on the existence of such a pool (41). The fibrils of DNA in the pool form a dilute plasm. Such a gel would provide the individual DNA-helices with just the right mechanical conditions to perform a rotational movement with minimum energy around their own axis ("speedometer cable" (29)), which is the basis for a number of proposed replication mechanisms (8).

The great similarity in fine structure and the identical behaviour towards the versene test are evidence for a very close structural relationship between the bacterial nucleus and the phage DNA-pool. All evidence is now against complicated, complete mitosis in bacteria, *i.e.* morphologically the homogeneous nucleoplasm, and chemically the continuous production of DNA even in synchronized cells (31). These facts lead us to postulate that the genetic material of bacteria may multiply following the same mechanism as phage. The nucleoid would be simply a pool containing one or several bacterial genomes in a form similar to vegetative phage. To explain the genetic continuity of the bacteria together with the fact that during division extremely few (less than 1 per cent) abnormal, non-viable cells are segregated (35), we have to assume the existence either of only one linkage group or, when more linkage groups exist, of a high number of identical strands. Polyteny is easier to accord with the continuous synthesis of DNA and would also help greatly to understand some of the complicated segregation patterns found in the offspring of conjugated bacteria (4, 28) and the delay in appearance of phenotype in the case of some mutations. The division of a nucleoid would be a random separation into two daughter nucleoids each containing at least one complete genome.

At first glance, irradiation analyses would speak against a polyteny, since the survival curves of bacteria are mostly of the one-hit type (44), most simply explained by the existence of only one sensitive target. But here again, the similarity with the phage pool gives a contrary indication: the phage pool, indeed, also behaves as one unit, even when it already contains as much as 20 to 40 phage equivalents of DNA; the survival of phage production after x-irradiation or after decay of integrated P^{32} also presents a curve of the one-hit type (39). These observations would indicate that the interpretations of radiobiological data are not yet consistent among themselves.

The proposed hypothesis for the division of the bacterial nucleoid could even be extended to the division of the chromosomes in higher cells: these chromosomes are probably polytenic too, and again the mechanism of the repartition of the genome-strands between the daughter chromosomes is not yet known. Nothing then prevents us from considering the bacterial nucleoid as one single multistranded chromosome. The main difference between chromosomes would lie in the organization and the moiety which is not DNA. The high water content and consequently the low density of the bacterial nucleoids could be explained by the absence of protein and histones, and perhaps also by the absence of a supercoiling.

To start with the simplest chemical expression of genetic information as given by virus, and to proceed progressively to more and more complicated systems containing greater proteins and substances like ribonucleoproteins may be a rewarding approach to an understanding of the nuclei of higher cells.

References

1. Adams, M. H., *in* Methods in Medical Research, (J. H. Comroe, Jr., editor), Chicago, The Year Book Publishers, Inc., 1950, **2**, 1.

2. Ames, B. N., Dubin, D. T., and Rosenthal, S. M., *Science*, 1958, **127**, 814.
3. Anderson, T. F., *Cold Spring Harbor Symp. Quant. Biol.*, 1953, **18**, 197.
4. Anderson, T. F., and Mazé, R., *Ann. Inst. Pasteur*, 1957, **93**, 194.
5. Bahr, G. F., *Exp. Cell Research*, 1954, **7**, 457.
6. Bonifas, V., and Kellenberger, E., *Biochim. et Biophysica Acta*, 1955, **16**, 330.
7. Cohen, S. S., *J. Biol. Chem.*, 1948, **174**, 281.
8. Delbrück, M., and Stent, G. S., *in* The Chemical Basis of Heredity, (W. D. McElroy and B. Glass, editors), Baltimore, The Johns Hopkins Press, 1957, 699.
9. De Robertis, E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 785.
10. Doermann, A. H., *J. Bact.*, 1948, **55**, 257.
11. Gale, E. F., and Folkes, J. P., *Biochem. J.*, 1953, **53**, 493.
12. Grell, K. G., and Wohlfarth-Bottermann, K. E. *Z. Zellforsch.*, 1957, **47**, 7.
13. Hahn, F. E., Schaechter, M., Ceglowski, W. S., Hopps, H. E., and Ciak, J., *Biochim. et Biophysica Acta*, 1957, **26**, 469.
14. Haller, G. de, unpublished results.
15. Herriott, R. M., and Barlow, J. L., *J. Gen. Physiol.*, 1952, **36**, 17.
16. Hershey, A. D., *J. Gen. Physiol.*, 1953, **37**, 1.
17. Hershey, A. D., and Melechen, N. E., *Virology*, 1957, **3**, 207.
18. Hook, A. E., Beard, D., Taylor, A. R., Sharp, D. G., and Beard, J. W., *J. Biol. Chem.* 1946, **165**, 241.
19. Kellenberger, E., in Symposium Bacterial Cytology, Rome, Fondaziône Emanuele Paterno, 1953, 45.
20. Kellenberger, E., *Experientia*, 1956, **12**, 282.
21. Kellenberger, E., and Arber, W., *Z. Naturforsch.*, 1955, **10b**, 698.
22. Kellenberger, G., and Kellenberger, E., *Virology*, 1957, **3**, 275.
23. Kellenberger, E., and Ryter, A., *Schweiz. Z. allg. Path. u. Bakt.*, 1955, **18**, 1122.
24. Kellenberger, E., and Ryter, A., *Experientia*, 1956, **12**, 420.
25. Kellenberger, E., Schwab, W., and Ryter, A., *Experientia*, 1956, **12**, 421.
26. Kellenberger, E., and Séchaud, J., *Virology*, 1957, **3**, 256.
27. Kellenberger, E., Séchaud, J., and Ryter, A., to be submitted to *Virology*.
28. Lederberg, J., *Proc. Nat. Acad. Sc.*, 1957, **43**, 1060.
29. Levinthal, C., and Crane, H. R., *Proc. Nat. Acad. Sc.*, 1956, **42**, 436.
30. Luria, S. E., and Human, M. L., *J. Bact.*, 1950, **59**, 551.
31. Maaløe, O., *in* The Bacteria, (R. Y. Stanier and I. C. Gunsalus, editors), New York, Academic Press, Inc., **2**, chapter 3, in press.
32. Maaløe, O., Birch-Andersen, A., and Sjöstrand, F. S., *Biochim. et Biophysica Acta*, 1954, **15**, 12.
33. Mudd, S., Hillier, J., Beutner, E. H., and Hartman, P. E., *Biochim. et Biophysica Acta*, 1953, **10**, 153.
34. Murray, R. G. E., Gillen, D. H., and Heagy, F. C., *J. Bact.*, 1950, **59**, 603.
35. Powell, E. O., *J. Gen. Microbiol.*, 1958, **18**, 382.
36. Ris, H., *in* The Chemical Basis of Heredity, (W. D. McElroy and B. Glass, editors), Baltimore, The Johns Hopkins Press, 1957, 23.
37. Robinow, C. F., *Bact. Rev.*, 1956, **20**, 207.
38. Ryter, A., and Kellenberger, E., *Z. Naturforsch.*, in press.
39. Stent, G. S., *in* Advances in Virus Research, (K. M. Smith and M. A. Lauffer, editors), New York, Academic Press, Inc., 1958, **5**, 95.
40. Tomizawa, J. I., and Sunakawa, S., *J. Gen. Physiol.*, 1956, **39**, 553.
41. Visconti, N., and Delbrück, M., *Genetics*, 1953, **38**, 5.
42. Williams, R. C., and Fraser, D., *J. Bact.*, 1953, **66**, 458.
43. Wisseman, C. L., Jr., Smadel, J. E., Hahn, F. E., and Hopps, H. E., *J. Bact.*, 1954, **67**, 662.
44. Zelle, M. R., and Hollaender, A., *in* Radiation Biology, (A. Hollaender, editor), New York, McGraw-Hill Book Company, Inc., 1955, **2**, 365.

Explanation of Plates

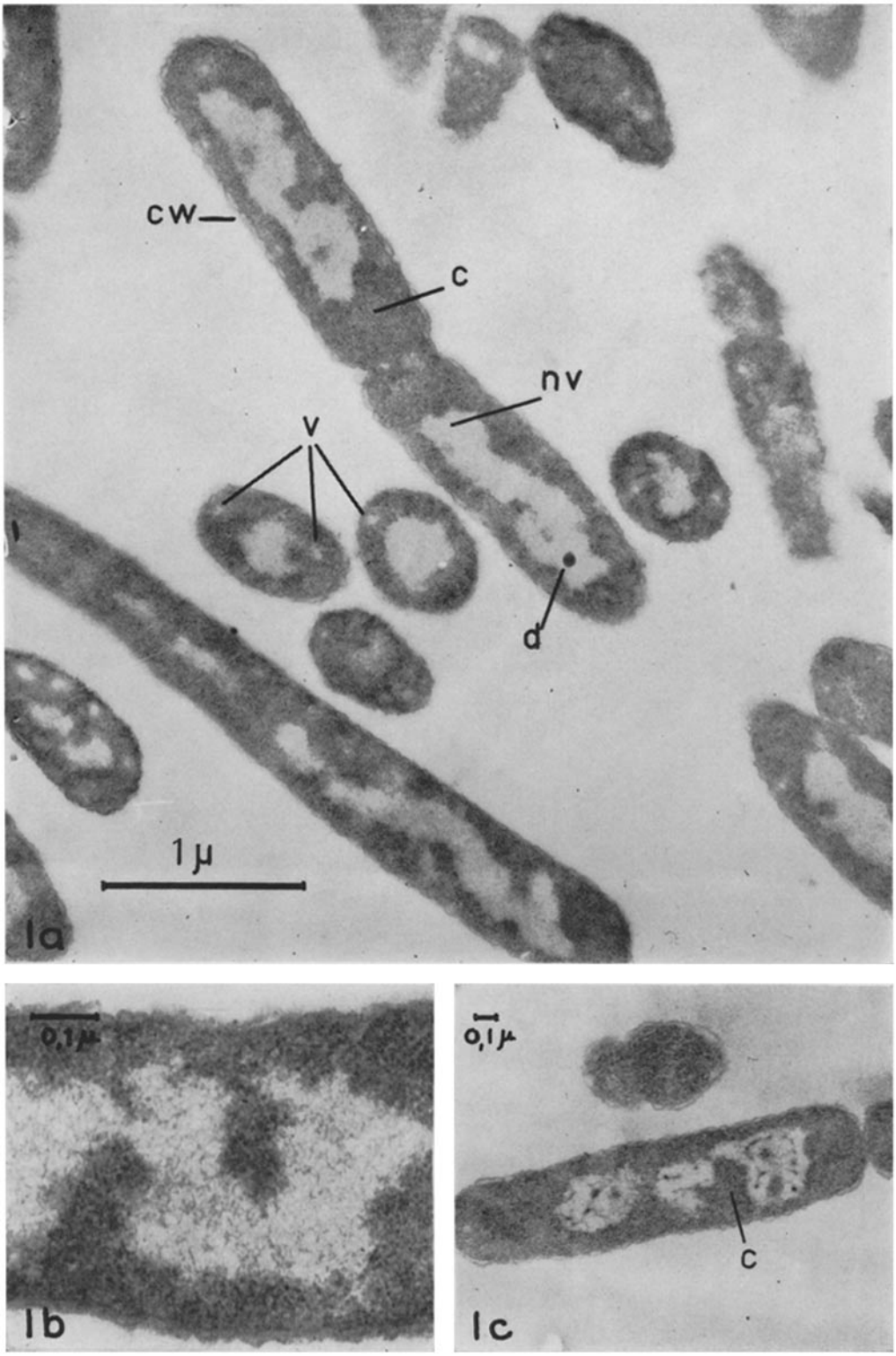
PLATE 330

FIG. 1 *a*. *E. coli* K12 S in exponential growth ($2 \cdot 10^8$ cells/ml.), cultivated in tryptone, fixed under the standard conditions, and embedded in vestopal W. × 30,000.

The nuclear vacuoles (*nv*) present variable forms, but all are filled with a fine and homogeneous nucleoplasm. The cytoplasm (*c*) is granulated and homogeneous. The cell wall (*cw*), a little detached from the cytoplasm, is clearly visible. (*d*) is a dirt particle and (*v*) are small cavities in the cytoplasm, frequently encountered but of as yet unknown origin and function.

FIG. 1 *b*. Detail of nucleoplasm. It is formed of thin filaments disposed as if a network. No nuclear membrane is visible. × 100,000.

FIG. 1 *c*. Versene test applied to the same bacteria as those of Fig. 1 *a*: the fine structure of the nucleoplasm has been destroyed by the versene treatment. Nucleoplasmic filaments have been collapsed and appear coarse and irregular. The granular structure of the cytoplasm remains unchanged (*c*). × 30,000.



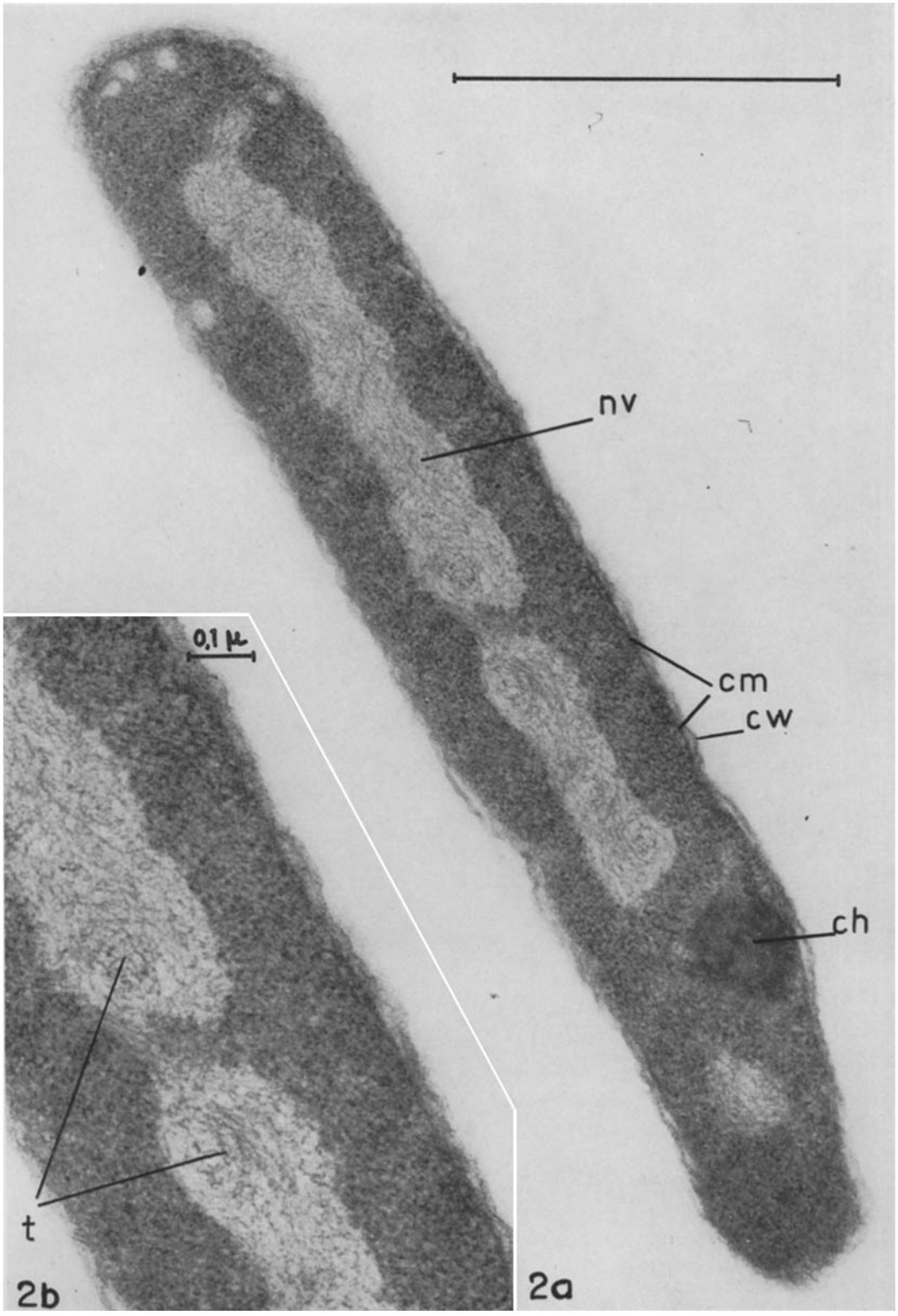
(Kellenberger *et al.*: DNA-containing plasms. II)

PLATE 331

FIG. 2 *a*. *E. coli* K12 S starved in 0.5 per cent NaCl solution pH 7 for 1 hour, fixed under the standard conditions, and embedded in vestopal W. × 60,000.

The starvation has produced the elongation of the nucleoid (*nv*). The nucleoplasm is composed of thin filaments. The cytoplasm is granulated and similar to that of the normal cell. A dense body, probably corresponding to a chondrioid (*ch*) is visible in cells grown under these culture conditions (for discussion see (38)), and the "layered" structure of the cell wall (*cw*) is clearly visible, as is the cytoplasmic membrane (*cm*).

FIG. 2 *b*. Higher magnification of the same bacterium of Fig. 2 *a* (× 100,000), showing better the parallel arrangement of the nucleoplasmic filaments. When this arrangement is transversely cut, it appears formed of many points (*t*).



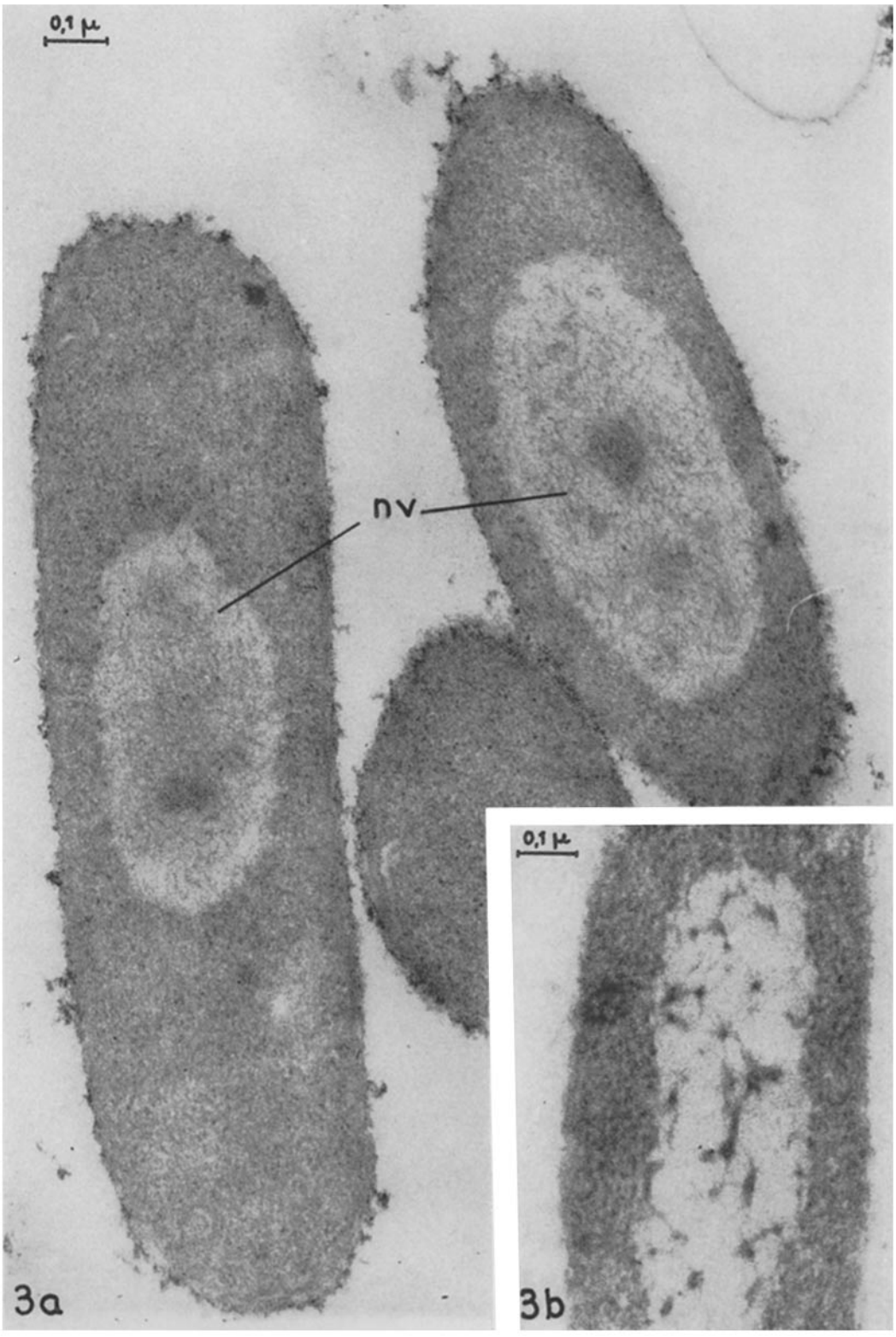
(Kellenberger *et al.*: DNA-containing plasms. II)

PLATE 332

FIG. 3 *a*. *E. coli* B cultivated in tryptone to which 25 γ/ml. of chloramphenicol were added when the bacteria had reached a concentration of $3 \cdot 10^8$ cells/ml. After 2½ hours, the bacteria were fixed under the standard conditions and embedded in vestopal W. × 90,000.

Bacteria show the typical response to this treatment, namely the vesicular form of the nucleoid (*nv*). The nucleoplasm is composed of thin filaments very similar to those seen in Figs. 1 and 2.

FIG. 3 *b*. Detail of the nucleoid of the same bacteria as Fig. 3 *a*, but submitted to the versene test, which again is positive. × 90,000.



(Kellenberger *et al.*: DNA-containing plasms. II)

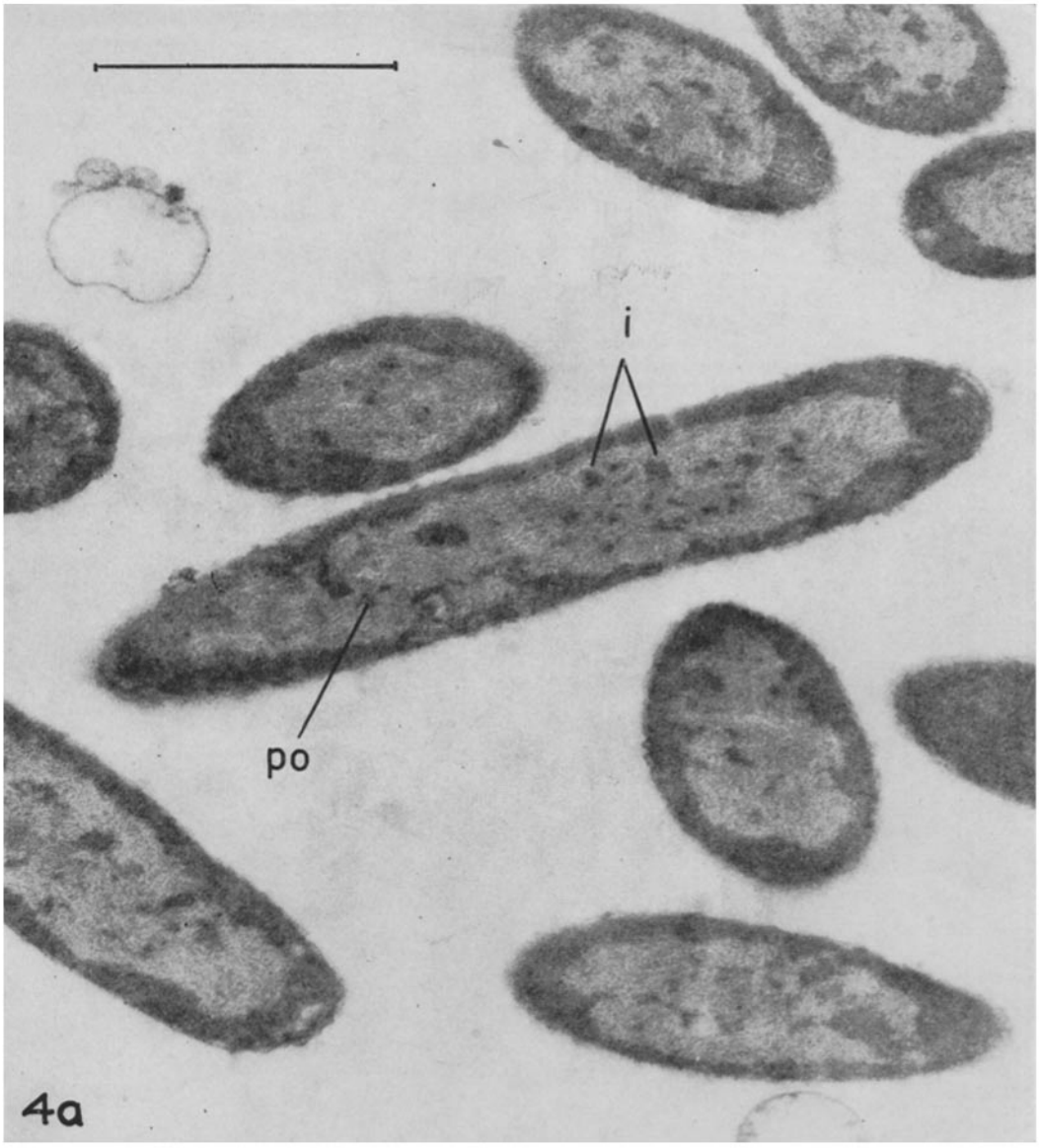
PLATE 333

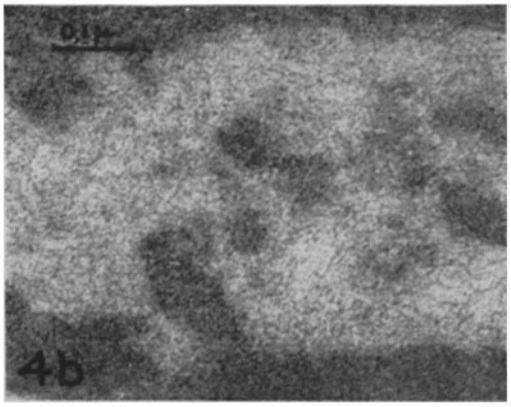
FIG. 4 *a*. *E. coli* B infected by about 3 T2 phages per bacterium, and 25 γ/ml. of chloramphenicol added 8 minutes after infection. The bacteria were fixed under standard conditions 75 minutes after this addition and embedded in vestopal W. × 30,000.

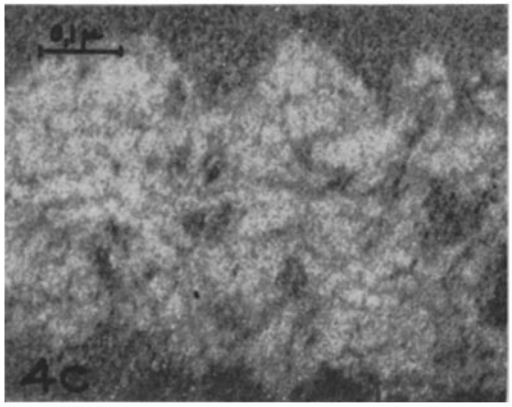
The giant pool of DNA (*po*) fills nearly the whole cell. The pool is constituted of thin filaments. The denser islets (*i*) in the pool are cytoplasmic protrusions transversely cut. The cytoplasmic granulation is not so visible as under other conditions of culture.

FIG. 4 *b*. Detail of the pool. × 100,000. Its aspect and structure are very similar to those of the bacterial nucleoplasm.

FIG. 4 *c*. The same as 4 *a* and *b*, but submitted to the versene test: coarse aggregation of the filaments. × 100,000.







(Kellenberger *et al.*: DNA-containing plasms. II)

PLATE 334

FIG. 5 *a* to *d*. *E. coli* B infected by T2 phage, fixed 30 minutes after infection under the standard conditions, and embedded in vestopal W. × 50,000.

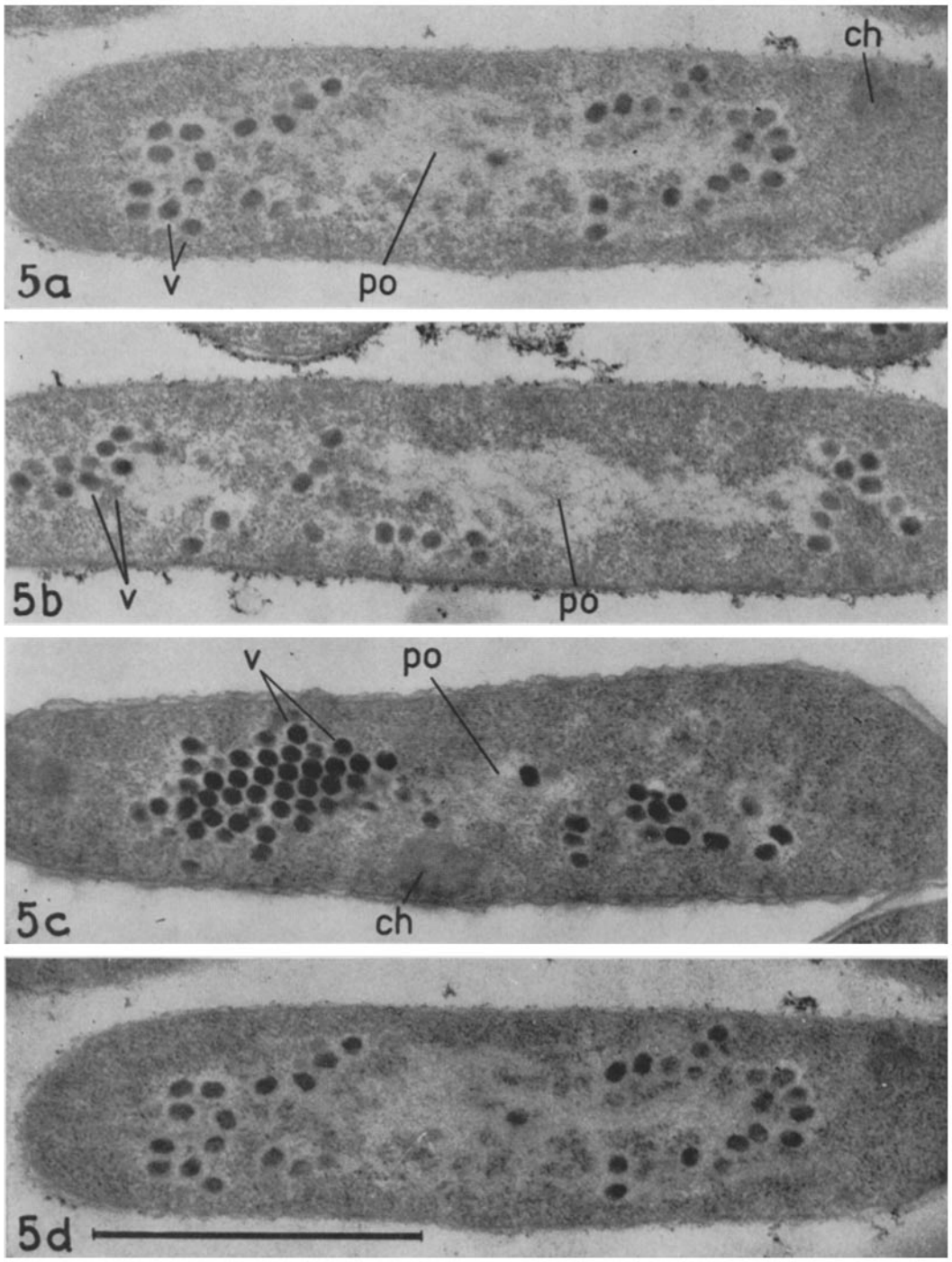
Mature virus (*v*) are clearly visible. Their polyhedral heads are well preserved. The pool (*po*) is composed of thin filaments, but it is more dilute than that of chloramphenicol-treated cells. The dense bodies appearing in the cytoplasm are probably the chondrioids (*ch*).

Figs. 5 *a* to *c* differ only in the washing bath between the same standard fixation and the dehydration: (*a*) is treated 1 hour in buffer containing 0.1 M Ca^{++}, (*b*) is treated for 2 hours in a 1 per cent solution of $La(NO_3)_3$ in buffer, (*c*) is treated for 2 hours in a 0.5 per cent solution of uranyl-acetate in buffer (pH 5).

All these micrographs were photographically treated in the same way, until the background appeared of the same grey shade.

FIG. 5 *d* is the same as Fig. 5 *a*, but enlarged on a photographic paper 2 grades harder.

These four pictures show that the lanthanum and especially the uranyl ions increase the contrast not only of the phages, but also of the bacterial cytoplasm, since photographic augmentation of contrast for a bacterium not treated with uranyl-acetate gives an image very similar to that of a treated cell.



(Kellenberger *et al.*: DNA-containing plasms. II)