*OBSERVATIONS ON THE SHAPE OF THE 50S RIBOSOMAL SUBUNIT**

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Communicated by Bernard D. Davis, September 14, 1968

Information on the morphology of ribosomal subunits has lagged far behind knowledge of their chemistry. Since the studies by Hall and Slayter[1] and Huxley and Zubay[2] on bacterial ribosomes, there have been few reports on structure. Hart[3] found parallel grooves in shadowed preparations of 50*S* subunits, Nanninga[4] noted a central depression in CsCl-treated particles, and several investigators[2, 4, 5] observed that some 50*S* subunits had polygonal outlines.

In the present investigation 50*S* subunits were examined to determine if there were any features of these particles which could be readily identified by the negative staining method. A model is proposed which accounts for features seen in various views: a crescent shape, a slit, and a projection ("nose") on top of a rounded base.

Materials and Methods.—Preparation of ribosomal subunits: Ribosomes were prepared from frozen log phase cells of *Escherichia coli* B or MRE 600[6] by alumina grinding. The cell paste was taken up in 2 vol of 10 mM tris-HCl, pH 7.6, 10 mM $MgCl_2$, and 50 mM KCl (buffer A) to which 1 μg/ml of DNase (Worthington, electrophoretically pure) was added. After centrifugation at 15,000 *g* for 15 min and 30,000 *g* for 30 min, the supernatant was centrifuged at 100,000 *g* for 2 hr. The pellet containing the 70*S* ribosomes was resuspended in buffer A and layered over 5 ml of the same solution containing 0.9 *M* sucrose (Mann, enzyme grade) and 0.5 *M* NH_4Cl. After centrifugation at 150,000 *g* for 4 hr, a clear pellet was recovered and resuspended in buffer A. The suspension was then stored at −15°C, or dialyzed against 10 mM tris-HCl, pH 7.6, 0.3 mM $MgCl_2$, and 50 mM KCl (buffer B) for at least 4 hr to dissociate the 70*S* ribosomes into subunits.

The 50 and 30*S* subunits were prepared by centrifugation of the dissociated 70*S* ribosomes in a 5–20% sucrose gradient containing buffer B (6.5 hr, 4°C, 25,000 rpm, Spinco SW25 rotor). The 50 and 30*S* regions of the gradient were separately collected and the sucrose was removed by dialysis against buffer B. The subunits were concentrated by centrifugation at 150,000 *g* for 6 hr and resuspended in buffer B. They were further purified by one or two successive fractionations in a similar sucrose gradient and 10 mM $MgCl_2$ was then added. The fractions were dialyzed against buffer A, concentrated by centrifugation, resuspended in buffer A, and stored at −15°C. The 50 and 30*S* subunits so prepared presumably consisted of "derived" subunits together with some "native" subunits.

Samples of frozen purified 50*S* subunits provided by Drs. T. Staehelin, R. E. Monro, and M. Nomura were also examined, as were unfrozen 50*S* subunits prepared from freshly grown cells of MRE 600. Negatively stained particles from all of these preparations had apparently similar morphology.

The purity of samples of 50 and 30*S* subunits was determined by centrifugation in a 5–20% sucrose gradient in buffer B (SW41 rotor, 40,000 rpm, 4°C, 3.5 hr). Cross contamination of the 50 and 30*S* preparations was estimated to be less than 3%.

Electron microscopy: Ribosomes were examined by negative staining with or without prior fixation. A droplet containing the particles, at a concentration of 0.1–0.4 mg/ml, was put onto a fenestrated ("holey") grid,[2] and the grid was rinsed with buffer A or B and again briefly with the stain (1% uranyl formate, pH 4.0; 1% ammonium molybdate, pH 7.0; or 1% uranyl oxalate, brought to pH 6.8 with ammonia[7]). All stains contained 0.1 mM $MgCl_2$, although its value was not established. The grid was then blotted at

the side and viewed in a Siemens Elmiskop 1 (double condenser with 200-μ aperture; 50-μ objective aperture; 80 kv; final instrumental magnification $\times$80,000).

Particles were seen most clearly when they were suspended in stain stretched across holes. Many holes were broken, however, and the ribosomal subunits were often markedly distorted. Particle size and shape were least variable when the stain was stretched across a hole without any break.

For fixation, a suspension of particles was diluted about 10 times into a solution containing formaldehyde (0.12%, in 15 mM each of sodium acetate and sodium 5,5'-diethylbarbiturate, pH 6.6) for 1–2 hr at 0°. All particles shown in the illustrations were so fixed, unless otherwise noted.

Results.—50S subunits: Most of the particles were seen in micrographs in one of three distinct views: crescent, nose, or slit. Examples of each are shown in Figures 1–3 and 4*A* (particles *c*, *n*, and *s*).

In preparations negatively stained with uranyl formate, crescents were often asymmetric, with one branch thinner than the other. In the nose view, the subunit almost always showed a difference in density between a rounded base and a less dense, somewhat triangular projection extending above the base. A number of particles in this view also showed a small but distinctive notch in the base, directly opposite the apex of the nose (Fig. 3*B*). The slit view was found less often than crescents or noses. The apparent size of the subunits often varied from one area of the grid to another, perhaps because particles were compressed or stretched unevenly, or because the depth of penetration of the negative stain varied.

In preparations negatively stained with ammonium molybdate, crescents and slits were commonly found. Nose views were less frequent, with little contrast between the nose and the surrounding stain (Figs. 2*A*, 3*B*). In the slit view, the stain was often denser in one half than in the other (Fig. 3*C*). When stained with uranyl oxalate rather than with uranyl formate, the subunits were less distinctly outlined and noses were seen much less frequently than crescents and slits (Fig. 2*B*).

30 and 70S particles: In samples of concentrated 30*S* subunits, particles often were aggregated, heterogeneous in size and shape, and indistinctly outlined (Fig. 3*G*). This variability may have been due to the instability of the subunits or to the particular method of preparation used. When 70*S* ribosomes were freshly dissociated by dilution into buffers containing 0.1 to 0.3 mM $MgCl_2$, the outlines of the 30*S* subunits, with or without fixation, were clearer (Fig. 4*A*, *B*). Often the 30*S* subunit was elongated, and a ridge or slight depression was found near the center. Some particles seemed foreshortened, as if tilted, and, infrequently, small circular particles were seen, which may have been 30*S* subunits on end.

Most 70*S* ribosomes, whether unfixed or fixed in 0.12 per cent formaldehyde for one hour, dissociated into subunits during the process of negative staining. Some held together and in some views a groove between the 50*S* and the 30*S* subunits was evident. Selected 70*S* ribosomes are shown in Figure 4*C*. Sometimes the larger subunit of these ribosomes resembled the crescent view of the isolated 50*S* subunits. In a few of the 70*S* ribosomes, there was a thin line of stain traversing the short axis of the 30*S* subunit. In general, 30*S* subunits ap-

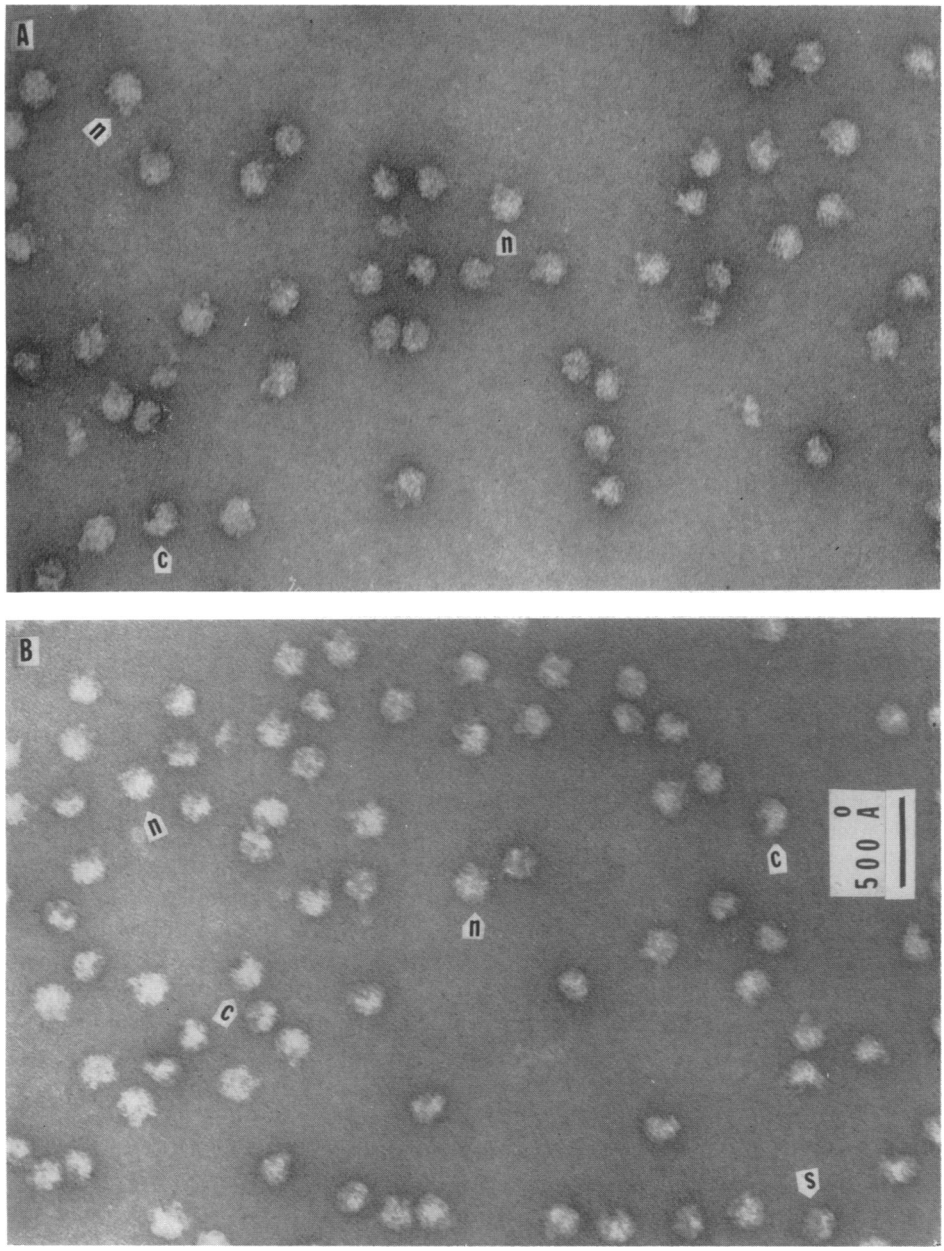

FIG. 1.—50*S* subunits negatively stained with uranyl formate. Crescent, nose, and slit views are indicated by *c*, *n*, *s*. ×225,000.

peared to be more uniform in shape when they were part of the 70*S* ribosomes than when they were isolated.

The exact conditions of fixation of the ribosomes and subunits were not critical, provided that low concentrations of formaldehyde were used. Particles that

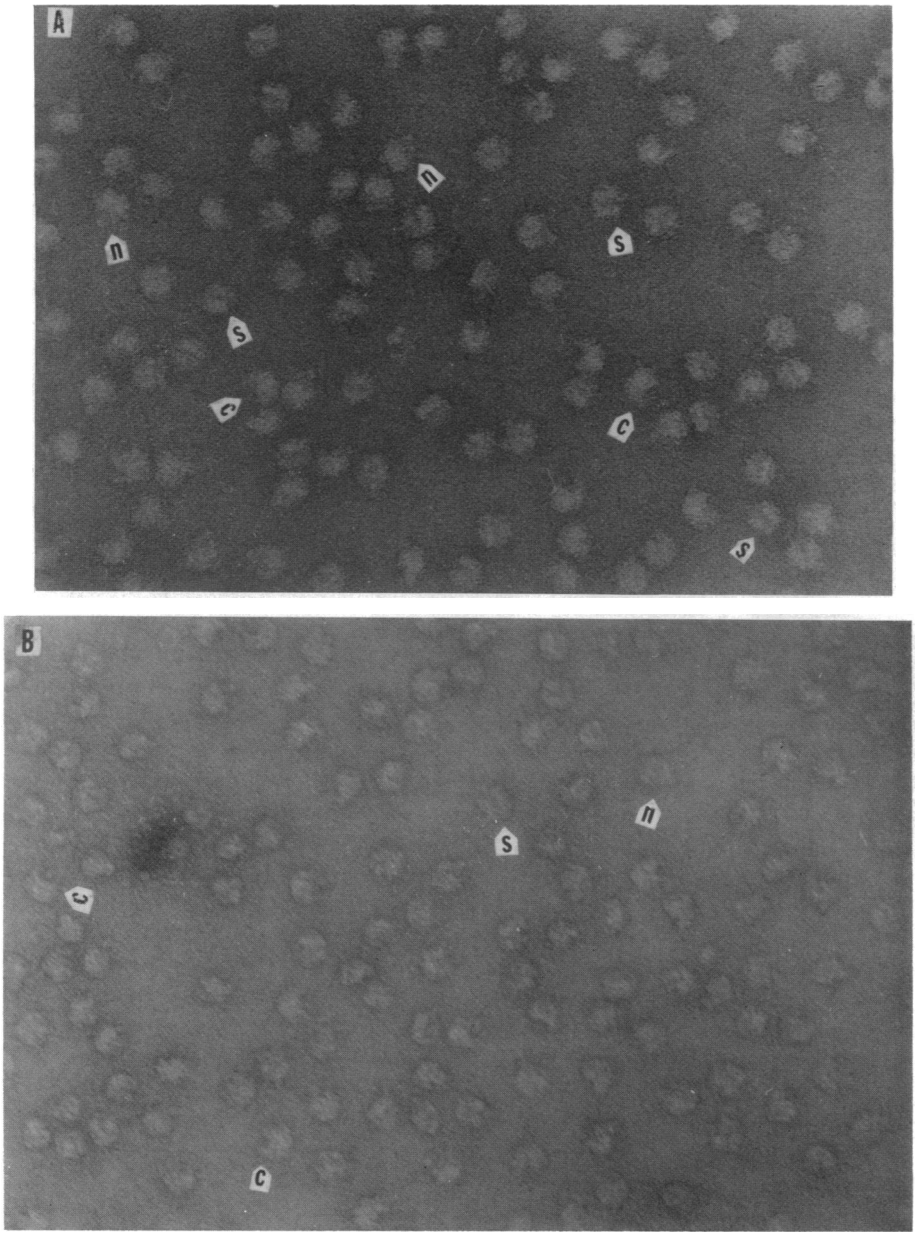

FIG. 2.—50S subunits negatively stained with ammonium molybdate (A, ×245,000) or uranyl oxalate (B, ×225,000).

were not fixed had shapes similar to those that were fixed, but seemed slightly fuzzier.[8]

Discussion.—When 50S subunits were negatively stained, they showed a number of characteristic features. Typical views of the particles displaying a cres-

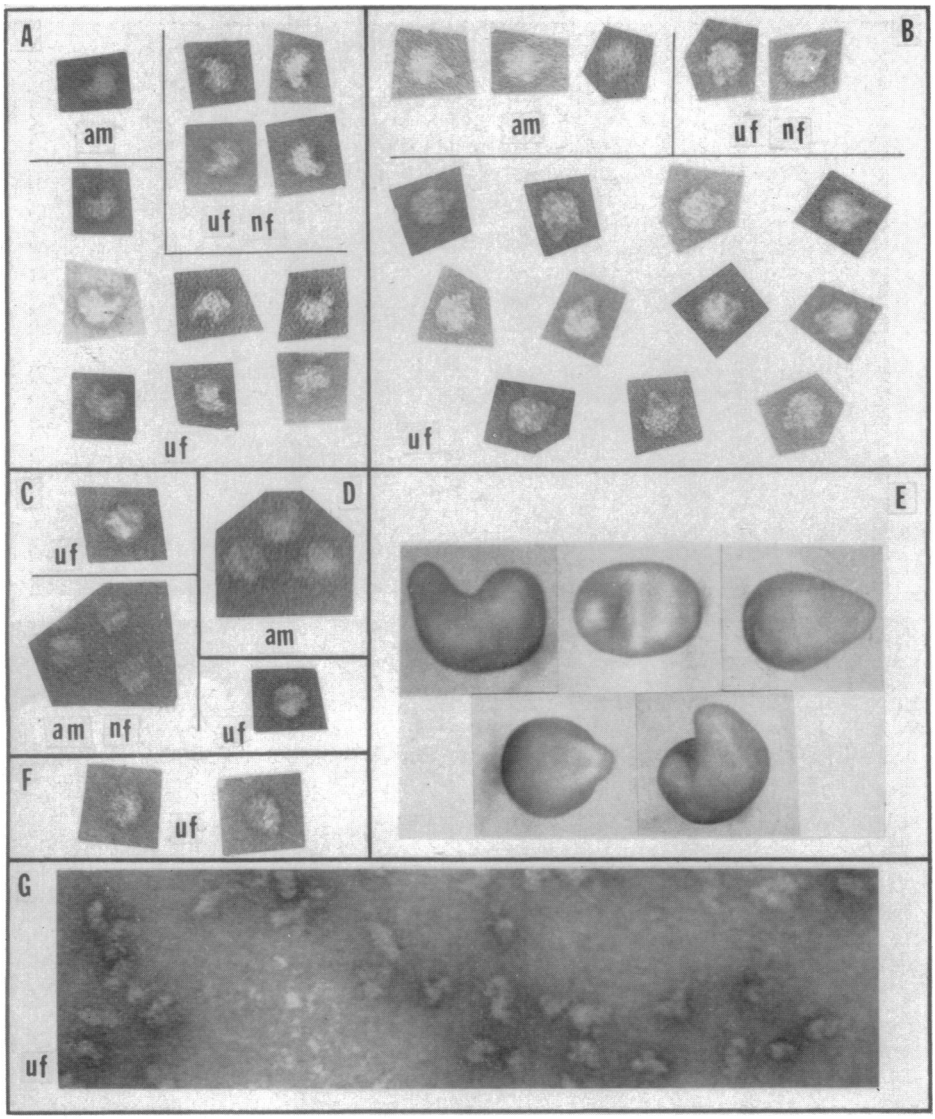

FIG. 3.—(*A–C*) Selected 50*S* subunits in the crescent, nose, or slit views. ×265,000. (*D*) Cluster of three 50*S* subunits showing crescent, nose, and slit views. ×375,000. (*E*) Photographs of clay model of 50*S* subunit, in crescent, slit, and nose views. Two other views are also shown. (*F*) Circular particles with central depression in preparation of 50*S* subunits, not accounted for by model. ×265,000. (*G*) Sample of purified 30*S* subunits. ×265,000.

All particles, except those designated as not fixed (*nf*), were fixed with 0.12% buffered formaldehyde and negatively stained with uranyl formate (*uf*), or ammonium molybdate (*am*).

cent shape, a nose, or a slit could be readily identified. It seems important to note that these features could be demonstrated not only with acid uranyl formate but with uranyl oxalate and ammonium molybdate at a pH close to neutrality.

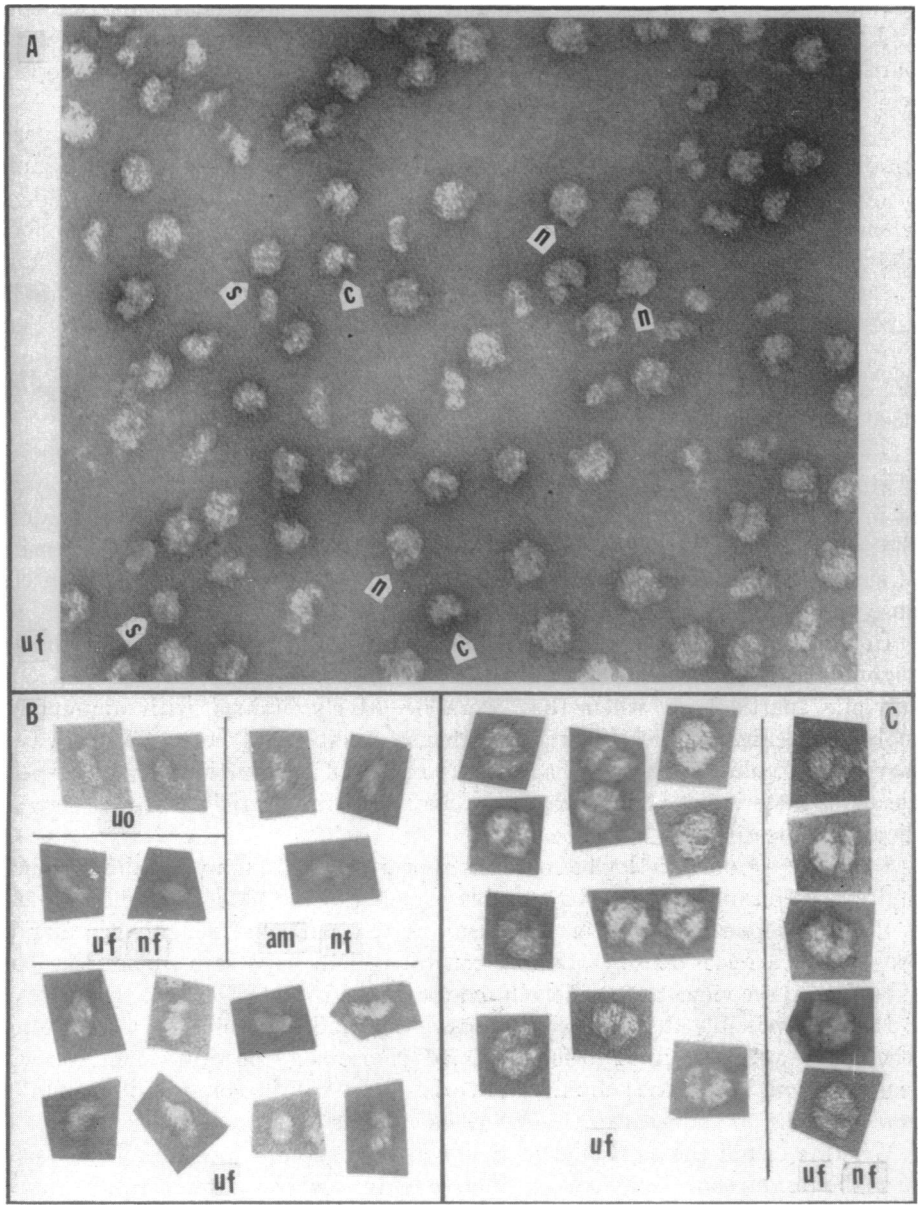

FIG. 4.—(*A*) Dissociated ribosomes: A sample of 70*S* ribosomes was dialyzed against buffer B, fixed, and negatively stained with uranyl formate. Crescent, nose, and slit views of 50*S* subunits are labeled *c*, *n*, *s*. Smaller particles are presumably 30*S* subunits. ×265,000.

(*B*) Selected 30*S* subunits, taken from micrographs of dissociated 70*S* ribosomes. ×265,000.

(*C*) Selected 70*S* ribosomes. ×265,000.

All particles, except those designated as not fixed (*nf*), were fixed with 0.12% buffered formaldehyde and negatively stained with uranyl formate (*uf*), uranyl oxalate (*uo*), or ammonium molybdate (*am*).

It has been assumed that the samples of 50*S* subunits were nearly homogeneous and that the crescent, nose, and slit views were different projections of the same particles. This uncertainty could probably be resolved by use of a microscope in which the grid can be tilted through an angle of 90°.

A tentative model of the 50*S* subunit was shaped from clay. When properly rotated, the model displays the crescent, nose, and slit views seen in micrographs of 50*S* subunits (Fig. 3*E*). The central slit or depression is apparently hidden when the model is turned over. Particles on the grid would be expected to look about the same if turned over, however, because they were suspended in stain covering both top and bottom surfaces, and the image formed in micrographs gives equal weight to details on both top and bottom. The proper way to look at the model is to imagine that one can look *through* it so that features on the back are given as much importance as those on the front. Only three orthogonal views need be considered, therefore, not six.[9]

Two other features of the model may account for the nonuniform distribution of stain in particles in certain views. When the nose view of the model is looked at in projection, the thickness is much greater in the region of the base than in the section producing the nose. The base would therefore have a lesser density of stain. This difference in density was often seen in particles oriented in the nose view (Figs. 1, 3*B*, 4*A*).

In the slit view of the model, the left and right halves are different, and an asymmetrical distribution of stain was sometimes found in slit views of 50*S* subunits, particularly when they were negatively stained with ammonium molybdate (Fig. 3*C*, *D*). The appearance of a slit might be explained in two ways: thickening of the stain across the center of the particle, or the greater electron scattering in regions where the surface of the particle was not perpendicular to the direction of the beam.

Some 50*S* subunits in the micrographs appeared to be roughly equidimensional, and can be accounted for by a suitable rotation of the model so that the nose is directly superimposed over the base. A few particles, roughly circular in shape with a more densely staining central region, were seen among the 50*S* subunits; these views are not accounted for by the model (Fig. 3*F*).

It seems possible that the shapes of the 50*S* and 30*S* subunits were altered when they were combined to form the 70*S* ribosome. No firm conclusion was reached about how these subunits were oriented in the ribosome, and no suitable model for the 30*S* subunit or the 70*S* ribosome was constructed.

Whether or not the particular model of the 50*S* subunit presented here is correct, the results show that some distinctive features of this subunit can be recognized in negatively stained preparations. These features may provide useful points of reference in further studies on the location of specific proteins of the 50*S* subunit or on the sites of attachment of transfer RNA.

This work was carried out in the laboratory of Dr. H. E. Huxley, to whom I am indebted for many helpful suggestions. Mrs. Robin George, Miss Rene Tankenoff, Mrs. Madeleine Böhni, and Thomas Lubin gave valuable assistance.

* Supported by the Medical Research Council, the Guggenheim Foundation, The Commonwealth Fund, the National Institutes of Health, and the National Science Foundation.

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[1] Hall, C. E., and H. S. Slayter, *J. Mol. Biol.*, **1**, 329 (1959).

[2] Huxley, H. E., and G. Zubay, *J. Mol. Biol.*, **2**, 10 (1960).

[3] Hart, R. G., these PROCEEDINGS, **53**, 1415 (1965).

[4] Nanninga, N., *J. Cell Biol.*, **33**, C1 (1967).

[5] Kiselev, N. A., and A. S. Spirin, in *Proceedings of the Third European Regional Conference on Electron Microscopy, Prague, 1964*, ed. M. Titlbach (Prague: Czechoslovak Academy of Sciences, 1965), vol. B, p. 39.

[6] Cammack, K. A., and H. E. Wade, *Biochem. J.*, **96**, 671 (1965).

[7] Mellema, J. E., E. F. J. van Bruggen, and M. Gruber, *Biochim. Biophys. Acta*, **140**, 180 (1967).

[8] Higher concentrations of formaldehyde (1–4%) or glutaraldehyde (0.5–2.5%) produced at least two changes in structure. First, the particles were considerably more heterogeneous in size and shape than those shown in Figs. 1 and 2. Although some crescents, noses, and slits could still be recognized, most particles did not have any obvious distinguishing features. Many particles, particularly the 70*S* ribosomes, showed a number of straight edges. Second, the particles showed a considerable increase in the amount of visible detail; their surfaces contained many pits and grooves. In a few particles the grooves were arranged in parallel, about 35–45 Å apart. It seems possible that these detailed structures were the result of distortion produced by the fixative, or else that the particles were partially degraded by the fixative and an underlying structure was revealed.

A wide range of conditions of fixation was studied, including formaldehyde, glutaraldehyde, osmic vapors, and combinations of these, at pH values from 6.0 to 7.6, for 30 min to several days. No conditions were found which produced a more homogeneous population of subunits than the mild fixation described earlier in the text. High concentrations of fixatives were clearly necessary, however, for maintenance of the outlines of the ribosomal particles when potassium phosphotungstate was used as a negative stain, as noted by Huxley and Zubay,[2] presumably because the phosphotungstate ion complexes with magnesium ions. With calcium phosphotungstate as a negative stain, no prior fixation was necessary.

[9] A better method for testing the validity of this model would be provided by the technique described by D. L. D. Caspar (*J. Mol. Biol.*, **15**, 365 (1966)), in which the model is embedded in plaster and examined by X-ray radiography.