double-stranded RNA (Table 2). Half of the label in double-stranded RNA is in viral RNA and half is in the complementary strand. Therefore, the ratio of viral to complementary RNA molecules is as 8.4 : 1.

22. S. Spiegelman, I. Haruna, I. Holland, G. Beaudreau, D. Mills, *Proc. Nat. Acad. Sci. U.S.* **54**, 919 (1965); I. Haruna and S. Spiegelman, *ibid.*, p. 579.
23. N. Zinder and S. Cooper, *Virology* **23**, 152 (1964).
24. S. Cooper and N. Zinder, *ibid.* **18**, 405 (1962).
25. R. Martin and B. Ames, *J. Biol. Chem.* **236**, 1372 (1961).
26. The Vogel-Bonner minimal medium (*24*) was supplemented with 0.1 percent Difco vitamin-free casamino acids.
27. H. Lodish and N. Zinder, *Biochem. Biophys. Res. Commun.* **19**, 269 (1965).

28. Supported by NSF grant GB-1730. We thank Drs. K. Horiuchi and C. Weissmann for helpful discussions, and also Dr. Weissmann for communication to us of unpublished results.

22 December 1965

Zonal Ultracentrifuge for the Separation of Ribosomal Subunits

Abstract. *Ribosomal subunits were prepared by means of the Anderson zonal ultracentrifuge. With this technique it is possible to separate more than 300 milligrams of subunits with complete resolution in a single run, as compared with about 2 milligrams with conventional rotors. Superior resolution is achieved in the zonal ultracentrifuge, affording possibilities for the detection and preparation of minor ribosomal components.*

The quantity of fractionated ribosomal subunits which may be conveniently prepared by the conventional method of zone centrifugation in swinging buckets or in fixed-angle rotors imposes a severe limitation to the study of the structure and the function of ribosomes. Our results show that with the Anderson zonal ultracentrifuge (*1*) the number of subunits prepared in a single run may be increased by a factor of at least 100, with resolution of remarkable quality.

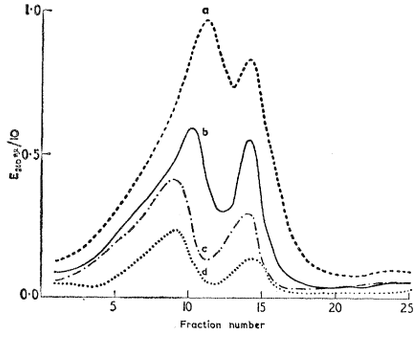

Fig. 1. The effect of loading on the fractionation of ribosomal subunits by zone centrifugation. The gradients of 10 to 25 percent sucrose with 0.001*M* tris, *p*H 7.6, were centrifuged for 19 hours at 25,000 rev/min (Spinco SW25 rotor) at 0°C. The direction of sedimentation is from right to left: (*a*) 2 mg; (*b*) 6 mg; (*c*) 8 mg; and (*d*) 16 mg.

Figure 1 shows how the quantity of dissociated reticulocyte ribosomes (*2, 3*) centrifuged in a single bucket of the Spinco SW25 rotor affects the quality of the separation between the subunits. Under these conditions it is apparent that mutual contamination of the two subunits occurs with a loading of more than 2 mg on the gradient. The conventional rotors of somewhat greater capacity which attain similar or greater centrifugal fields (such as Spinco SW 25.2, Spinco 30, MSE 40) do not substantially facilitate the preparation of subunits.

The capacity of the Anderson zonal ultracentrifuge rotor is 1760 ml. On this basis one would expect to be able to separate larger quantities of material. A second important difference between the zonal and conventional rotors is that, in the former, loading the sample at the beginning and unloading the gradient at the end of the run occur while the rotor is centrifuging at 5000 rev/min. Diffusion of the sample which may cause appreciable band broadening when the rotor is at rest (*4*) is therefore minimum.

A typical fractionation of 200 mg of reticulocyte ribosomal subunits is shown in Fig. 2. The sample (4 mg/3 ml) was applied in 0.1*M* sodium ethylenediaminetetraacetate (EDTA), *p*H 7.6, to a gradient, linear with respect to radius, of 10 to 25 percent sucrose containing 0.01*M* sodium acetate. The gradient was layered over a cushion of 35 percent sucrose, and the sample was layered with water. Centrifugation was carried out at 40,000 rev/min for 6 hours at 5°C. The gradient was then displaced by a concentrated sucrose solution and monitored at 290 mμ in a 1-cm flow cell with a Beckman DB recording spectrophotometer. Fractions (40 ml) were collected, and the refractive indexes were measured to determine the density. The recovery of ribosomes was found to be 182 mg as judged by the optical density pattern. Over 300 mg of dissociated ribosomes have been fractionated with complete resolution of the two subunits by this method.

The purity of the isolated subunits has been established within the limits of the analytical centrifuge (Spinco model E), ultraviolet optics being used. Furthermore, the RNA which may be isolated from the subunits is undegraded, the larger subunit yielding only 30*S* RNA and the smaller subunit 19*S* RNA, the values which have previously been attributed to the two species of reticulocyte ribosomal RNA (*2*).

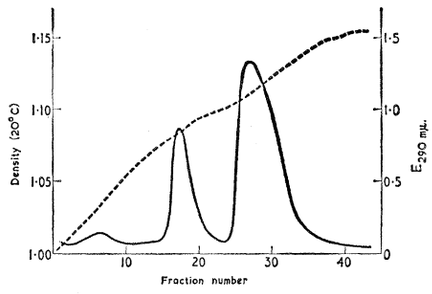

Fig. 2. Fractionation of 200 mg of rabbit reticulocyte ribosomal subunits. First a cushion of 35 percent sucrose and then the gradient was pumped into a Spinco B-IV zonal rotor at 3000 rev/min (modified Spinco Model L). The 200-mg sample of dissociated ribosomes was then layered onto the gradient at 5000 rev/min, and the sample was layered with water. The rotor was accelerated to 40,000 rev/min and the run was continued for 6 hours. The rotor was then decelerated to 5000 rev/min; the gradient was pumped out through a flow cell and the optical density was measured. All operations were carried out at 5°C. The direction of sedimentation is from left to right.

It is anticipated that the superior resolution of the subunits which may be obtained will facilitate the search for minor components (such as messenger RNA, and complexes of messenger and transfer RNA bound to the ribosomal subunits) which may be revealed by biological assays.

E. S. Klucis
H. J. Gould

Chester Beatty Research Institute, London, S.W.3, England, and *Biochemistry Department, University College, London, W.C.1*

References and Notes

1. N. G. Anderson, *Beckman Fractions* **1**, 2 (1965); *New Sci.* **24**, 732 (1964).
2. H. R. V. Arnstein, R. A. Cox, H. J. Gould, H. Potter, *Biochem. J.* **96**, 500 (1965).
3. H. J. Gould, H. R. V. Arnstein, R. A. Cox, *J. Mol. Biol.* **15**, 600 (1966).
4. M. K. Brakke, *Arch. Biochem. Biophys.* **107**, 388 (1964).
5. N. G. Anderson, personal communication.

6. We thank Drs. P. Alexander and H. R. V. Arnstein whose interest prompted this research, and Drs. K. S. Kirby and J. Hastings for discussions, K. Kanagalingham for analytical centrifuge runs and Miss S. Sharpe and K. Merai for technical assistance.

6 December 1965