measurement, due to imperfect knowledge of the refractive index in the small portion of the range included in the atmosphere, and due also to the unknown deformation of the earth crust. When the measurements are made over interplanetary distances, these uncertainties become much smaller than 1 part in 10^{10}. Second, the ranges involved, even though enormous when compared to anything with which we have any experience (three orders of magnitude above "moon-radar" ranges), are quite manageable with modern radio techniques. Indeed, it has been calculated that isotropic planetoid emissions of the order of 1 watt will yield more than adequate signal-to-noise ratios at the earth station. And third, comparison of the orbital data thus obtained with the orbital data of the planets would increase the accuracy of our determinations of interplanetary distances by several orders of magnitude.

This last point is the crux of the matter. Interplanetary distances are known today with an absolute accuracy of the order of 1 part in 10^3 only, owing to the poor degree of accuracy with which we know the parallax of the sun. On the other hand, interplanetary distances relative to each other are known with a much higher degree of accuracy, of the order of 1 part in 10^9. Thus, the availability, for a single orbit, of data known with an accuracy of the order of 1 part in 10^{10} would yield immediate knowledge of the other interplanetary distances with an accuracy of 1 part in 10^9. This means that a single successful planetoid experiment would increase the absolute accuracy with which interplanetary distances are known from a figure well below that realizable in laboratory measurements to a figure well above it. This means also that later and more elaborate planetoidal experiments will permit closer checks of the relativisitic gravitational correction, more accurate estimates of the distribution of large masses and of dust within the solar system, and so on. It even appears permissible to begin to speculate about the possibility of detecting spatial curvature or departures from the gravitational theory by means of these techniques.

It is also worth noting that if the experiments forecast here are successful, the standard meter, the wavelength of the green line of Hg^{198} and the sidereal second will play a secondary part in these measurements. The periods of the satellites and planets will be measured eventually in terms of (for instance) the cesium line period t_0; the orbital dimensions will be measured in terms of the wavelength of the cesium line λ_0; the product of the mass of a planet and the gravitation constant will be measured in terms of $\lambda_0^3 t_0^{-2}$, and so on. We shall have of course $\lambda_0 = ct_0$, by definition, but any determination of c in terms of "secondary" standards, such as the standard meter, the sidereal second, or the wavelength of the green line of Hg^{198}, will be of much lower accuracy than the measurement of planetary parameters for which λ_0 and t_0 will be the newly adopted primary standards.

MARCEL J. E. GOLAY
Philco Corporation,
Philadelphia, Pennsylvania

Note

1. A detailed and quantitative description of this technique has been published (M. J. E. Golay, "Interferometric rocket guidance," *Conf. Proc. PGMil of IRE, 2nd Natl. Conv.*, p. 182).

6 July 1959

High-Resolution Density Gradient Sedimentation Analysis

Abstract. The principle of stability for a sample layered in a density-gradient liquid column is discussed, and a method for separating ribonucleoprotein particles by means of sedimentation in the ultracentrifuge is described.

In the process of our studies of bacterial ribonucleoprotein particles (ribosomes), the need arose for a method of sedimentation analysis which would supply separated samples of the ribosomes of *Escherichia coli* (*1–3*). These particles have sedimentation constants of about 20, 30, 50, 70, and 100*S*. Adequate separation of these classes has been obtained by sedimentation at 105,000*g* through a density-gradient stabilized liquid column, use being made of the principle that the stabilizing density gradient must always exceed the inverted density gradient introduced by the sample. Since the method is mechanically simple and probably of general application, it is described separately in this report.

Density gradients are commonly applied to prevent mixing in liquid columns which are used for zone analysis by means of centrifugation or electrophoresis. However, this method has been limited to very small quantities of material, since the sample itself may introduce a region of density instability. So long as the density increases in the direction of the gravitational (or centrifugal) field, the gradient will exercise a stabilizing force against mixing which occurs as a result of mechanical disturbances or temperature gradients. If the density gradient is locally inverted due to the presence of a sample, the liquid containing the sample will stream through the less dense underlying layers. This process does not necessarily stop when the stream reaches a region of equal density, since the stabilizing solute (usually more rapidly diffusing than the sample) will diffuse into the stream and may continually reestablish a condition of instability.

The sample layer shown in Fig. 1*A* is initially stable. However, as soon as the sample is moved downward (or the stabilizing solute diffuses into the sample layer), the inverted density gradient will cause streaming. The inverted *density* gradient may be avoided if the sample is introduced (Fig. 1*B*) with a *concentration* gradient opposite to that of the stabilizing gradient, provided the inverted density gradient due to the sample itself is significantly less than the stabilizing density gradient.

Usually a maximum quantity of sample can be analyzed when both gradients are linear. The amount of sample which can be handled rises with the square of the width of the sample layer, since it is the *gradient* in density and not the maximum density of the sample which determines stability.

The instability of sharply defined sample layers has been previously recognized and offset (*4*) by stirring the sample layer to reduce the inverted gradient at the lower edge of the band. Stable inverted sample gradients of complex shape have been created through the use of mixing chambers (*5*). However, the large sample capacity and the simplicity of the inverted linear sample gradient have not previously been mentioned in the literature.

This principle has been applied successfully both for analysis by electrophoresis and for analysis by sedimentation in the ultracentrifuge. Since the latter application has been of great importance to our experiments on synthesis of (*1, 2*) and by (*6*) the ribosomes of *E. coli,* it is described here in detail.

A linear stabilizing density gradient of sucrose (20- to 5-percent) was prepared with a modification of the linear gradient mixing device of Bock and Ling (*7*), shown in Fig. 2. Such gradients are stable for many hours, and only moderate care need be taken in handling the tubes. The same device was then used to introduce the inverted sample gradient. For this purpose the left-hand chamber was loaded with 0.2 ml (for example) of buffer containing 5 mg of ribosomes per milliliter, and the right-hand chamber with 4-percent sucrose in the same buffer. The sudden step in sucrose concentration from 5 to 4 percent makes it possible to start the sample gradient without undue mixing. The stabilizing gradient can be reduced when the sample to be analyzed is small. Gradients of 10- to 3-percent sucrose

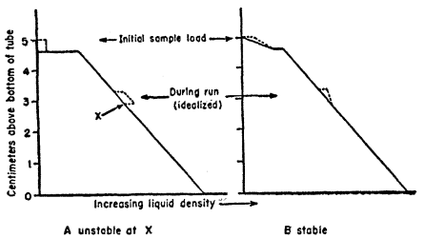

Fig. 1. Conditions for stability in a density gradient liquid column. Solid line, stabilizing gradient; dotted line, contribution due to a sample in process of analysis.

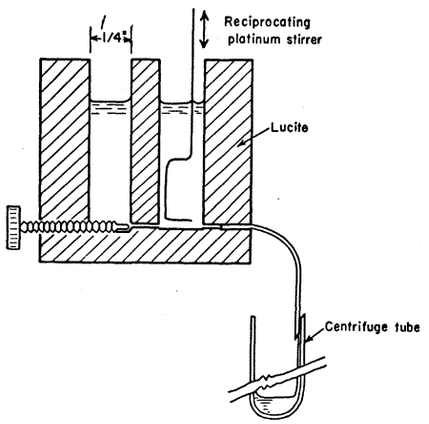

Fig. 2. Mixing chamber for the production of linear stabilizing gradients. The left chamber is filled with 2.4 ml of 5 percent sucrose, and the right, with 2.2 ml of 20-percent sucrose. After filling and after starting of the mixing motor, the center valve is opened and the exit tubing is turned down to touch the side of the centrifuge tube. The sucrose solution then runs down the wall of the tube. If the 4.6 ml are delivered in 10 to 15 minutes, the succeeding lighter solution floats on the underlying liquid with little mixing.

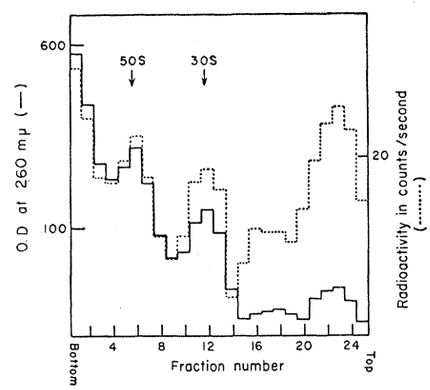

Fig. 3. Example of the use of density gradient stabilized sedimentation analysis for measurement of radioactivity incorporated into the smaller ribosomes during 1-minute exposure to $S^{35}O_4^{--}$. Solid line, optical density at 260 mμ of 0.2-ml samples diluted to 1.2 ml. Dotted line, trichloroacetic acid precipitable radioactivity.

have been used occasionally and are quite adequate.

This whole process was carried out in the cold room with solutions at 1° to 4°C. The tube was loaded into the precooled swinging bucket rotor (Spinco SW39) and centrifuged for the appropriate time. As quickly as possible at the end of the run, the tube was gently lifted out of the rotor and mounted in a device which perforated the bottom of the centrifuge tube with a hypodermic needle (ground to a short, double-sided point) located about 1 mm above the bottom of the tube. After removal of a piano wire which kept the hypodermic tubing clear and free of air bubbles, the contents of the tube were run out in 25 equal cuts by drop counting.

Figure 3 shows the result of use of this system for examination of the radioactivities of the smaller particles of *Escherichia coli* after a short period of incorporation of radioactive sulfate. A growing culture of *E. coli* was starved of sulfur for 30 minutes, and then $S^{35}O_4^{--}$ was added. After 1 minute, carrier $S^{32}O_4^{--}$, S^{32}-cystine, and S^{32}-methionine were added to displace the radioactivity of the rapidly-turning-over soluble proteins synthesized by the ribosomes (*6*). Fifteen seconds later the culture was suddenly chilled to 0°C, the cells were washed and broken, and the ribosomes were harvested in the preparative ultracentrifuge. The ribosome pellet was resuspended in appropriate buffer, and a sample was loaded in an inverted linear gradient layer, as described above. The tube was then centrifuged (37,000 rev/min, for 150 minutes) to pellet most of the 70 and 100*S* ribosomes in order to spread out the 20-to-50*S* region for a close examination of the smaller ribosomes.

The peaks in the diagram correspond closely with the peaks shown by the analytical centrifuge which was used to determine the sedimentation constants.

A series of experiments of this type (*2*) shows that the newly synthesized ribosomal protein and ribonucleic acid appear first in the smaller ribosomes (20, 30, 50*S*) and later in the larger ones (70, 100*S*).

A sample of the width discussed above has provided the best compromise between high resolution and a useful quantity for analysis, although the resolution could probably be improved to the limit set by diffusion if smaller samples and great care were used. For the example shown in Fig. 3, the full width (½ max.) due to diffusion alone would be about 1 mm or about ⅛ of the observed width. Since the width is due to causes other than diffusion, equivalent resolution could be obtained for objects very much smaller than the ribosomes. For example, serum globulin (molecular weight, 170,000, about 7*S*) would require about 12 hours of centrifugation, and the resulting diffusion width would be about 3 mm. For the preparation of large quantities of material, very broad sample gradients are useful. In the preparation of pure 30*S* particles from a mixture of 30 and 50*S* ribosomes, 2.4 ml of a very concentrated ribosome suspension are placed in the left chamber, and 2.4 ml of 20-percent sucrose, in the right chamber. In this way 10 to 20 mg of pure 30*S* particles may be obtained in a single run.

It is worth noting that, while the centrifugal force doubles from the top to the bottom of the tube in the swinging bucket rotor, the viscosity of 20-percent sucrose is about twice that of water. When correction is made for the density of the sucrose solution, it is found that the sedimentation velocity of ribosomes is very nearly constant throughout the length of the centrifuge tube.

R. J. Britten
R. B. Roberts
Department of Terrestrial Magnetism, Carnegie Institution of Washington, Washington, D.C.

References

1. R. B. Roberts, R. J. Britten, E. T. Bolton, in *Microsomal Particles and Protein Synthesis*, R. B. Roberts, Ed. (Pergamon, New York, 1958).
2. ———, *Carnegie Inst. Wash. Year Book No. 57* (1958); *Carnegie Inst. Wash. Year Book No. 58*, in press.
3. A. Tissières and J. D. Watson, *Nature* **182**, 778 (1958).
4. M. K. Brakke, *Arch. Biochem. Biophys.* **55**, 175 (1958).
5. H. Svensson, *Sci. Tools* **5**, 37 (1958).
6. K. McQuillen, R. B. Roberts, R. Britten, *Proc. Natl. Acad. Sci. (U.S.)* **45**, 1437 (1959).
7. R. M. Bock and N. S. Ling, *Anal. Chem.* **26**, 1543 (1954).

31 July 1959

Daily Light Sensitivity Rhythm in a Rodent

Abstract. Single 10-minute light periods can cause a phase shift in the rhythm of the daily locomotor activity of flying squirrels otherwise maintained in constant darkness. A daily rhythm of sensitivity to these standard light periods was found.

Recent investigations have demonstrated that many animals in isolation conditions exhibit a precise rhythm of locomotor activity which appears to be controlled by a "biological clock," characteristic in cycle length for each individual animal. Nocturnal rodents show cycles ranging from about 22 hours to about 25 hours in length for different individuals (*1*, *2*). These endogenous rhythms may be synchronized by clues