apparently restricted to the cotyledon as several analyses of leaf and root homogenates showed no activity. In experiments not shown here, it was found that the enzyme is present in extracts of acetone powders of eithers peanut or castor-bean cotyledons.

In order to test the specificity of the malate synthetase reaction, various compounds were compared with acetyl phosphate in the assay system of Table II. With acetyl pantetheine, acetyl glutathione and fluoroacetyl phosphate[5], 0.1, 0.4, and 0.1 μmoles glyoxalate disappeared. The data with fluoroacetyl phosphate and acetyl pantetheine are within experimental error while the apparent activity with acetyl glutathione may have been due to liberation of free thiol by a thiolesterase in the extract, with subsequent non-enzymic condensation with glyoxalate. To test this point, the reaction was followed by disappearance of thiol ester at 232 mμ. As shown in Fig. 1 neither acetyl pantetheine nor acetyl glutathione are reactive in the malate synthetase reaction and the extract does contain acetyl glutathione thiolesterase. These experiments further establish that acetylCoA is the reactive component and that both glyoxalate and enzyme are required for the reaction. In similar spectrophotometric experiments it was found that both propionylCoA and butyrylCoA are not active, indicating complete specificity of the reaction for acetylCoA.

Two types of experiments were performed to test the reversibility of the reaction (a) incubation of malate, CoA, and enzyme with hydroxylamine and analysis for acethydroxamic acid (b) incubation of malate, CoA and enzyme with a system of removal of acetylCoA (transacetylase and arsenate) and analysis for glyoxalate. The negative results obtained in both experiments indicate that the equilibrium is far in the direction of malate and that detection of reversibility will require an extremely sensitive assay.

Biological Sciences Branch, Agricultural Marketing Service, U.S. Department of Agriculture, Beltsville, Md. (U.S.A.)

ABRAHAM MARCUS
JAMES VELASCO

[1] D. T. O. WONG AND S. J. AJL, *J. Am. Chem. Soc.*, 78 (1956) 3230.
[2] H. L. KORNBERG AND N. B. MADSEN, *Biochim. Biophys. Acta*, 24 (1957) 651.
[3] H. L. KORNBERG AND H. BEEVERS, *Biochim. Biophys. Acta*, 26 (1957) 531.
[4] C. BRADBEER AND P. K. STUMPF, *J. Biol. Chem.*, 234 (1959) 498.
[5] A. MARCUS AND W. B. ELLIOTT, *J. Am. Chem. Soc.*, 80 (1958) 4287.
[6] T. E. FRIEDEMANN AND G. E. HAUGEN, *J. Biol. Chem.*, 147 (1943) 415.
[7] F. LIPMANN AND L. C. TUTTLE, *J. Biol. Chem.*, 159 (1945) 21.
[8] E. J. SIMON AND D. SHEMIN, *J. Am. Chem. Soc.*, 75 (1953) 2520.

Received August 13th, 1959

Biochim. Biophys. Acta, 38 (1960) 365–367

A method for plotting ultracentrifuge diagrams for polydisperse systems

The results of ultracentrifugal analyses, as generally recorded photographically, show a plot of the rate of change of refractive index (dn/dx) *versus* the distance from the center of rotation (x). To show changes occurring with time, several pictures are necessary. We therefore decided to examine the alternate possibility of using a plot of dn/dx *versus* time at one value of x since the results of one ultracentrifugal analysis may thus be presented in one picture. This is of some advantage where, by the usual

method, a number of diagrams from each analysis would be necessary to show both fast and slow peaks and several rows of diagrams would be needed for comparison of different samples. Results obtained by the usual method may be confusing since it is sometimes difficult to intercompare different runs, especially where the time intervals are not uniform.

A swing-out mask with a 0.03-inch vertical slit was installed close to the focal plane of a Spinco Model E analytical ultracentrifuge. The photographic plate was moved 0.05 inch every 2 min by a short-shift assembly. Two identical 12-mm 4° ultracentrifuge cells were used, one containing the solution to be analyzed, the other the solvent to provide a reference base line. Small differences between cells may be compensated by alternating the two solutions between the cells in consecutive runs.

Less exposure was needed to record the position of the base line than the experimental pattern. In these experiments the amount of light passed by the base-line cell was reduced by adding a small amount of the dye cloth red B to increase the extinction to about 0.25 at 580 mμ (1-cm light path), The Eastman Kodak Metallographic plates used were developed for 5 min in DK-19 developer. All runs were made at 56,100 rev./min and at 20° with a type D analytical rotor.

The first exposure was made, without the mask in place, as soon as the rotor had reached the desire speed. The mask was then moved into place and a second exposure made without moving the photographic plate. In this manner the position of the slit in relation to the meniscus and the reference hole was accurately located. The plate was then moved about 4 cm and exposures at 2-min intervals were begun.

The results of an ultracentrifuge run in which rat serum diluted 1:3 with buffer and dialyzed against buffer (pH 7.5, I 0.2) containing both phosphate and NaCl[1] were used are shown in Fig. 1A as a series of photographs* made at the indicated intervals. The results obtained on a similar preparation with the method described here are shown in Fig. 1B. In this experiment the level in the cell corresponding to the center of the slit was 0.535 cm from the meniscus.

The sedimentation coefficient of a given peak is inversely proportional to the time required for it to reach the slit level from the meniscus. The difficulty is in determining the true zero or starting time at which a given peak left the meniscus. In one series of experiments the centrifugation during acceleration was found by determining the area under a centrifugal force *versus* time curve for this period and calculating from

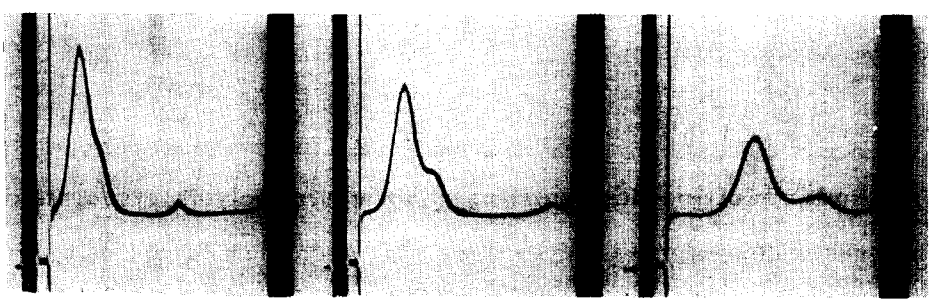

Fig. 1. A. Rat serum albumin diluted 1:3 with buffer (pH 7.5, I 0.2), photographed 20, 44, and 92 min after reaching 56,100 rev./min. Bar angle 55°, 20°C.

* Unfortunately the short shift assembly as supplied by the manufacturer moves the film the wrong direction. The photographic print must therefore be cut up into strips and reassembled.

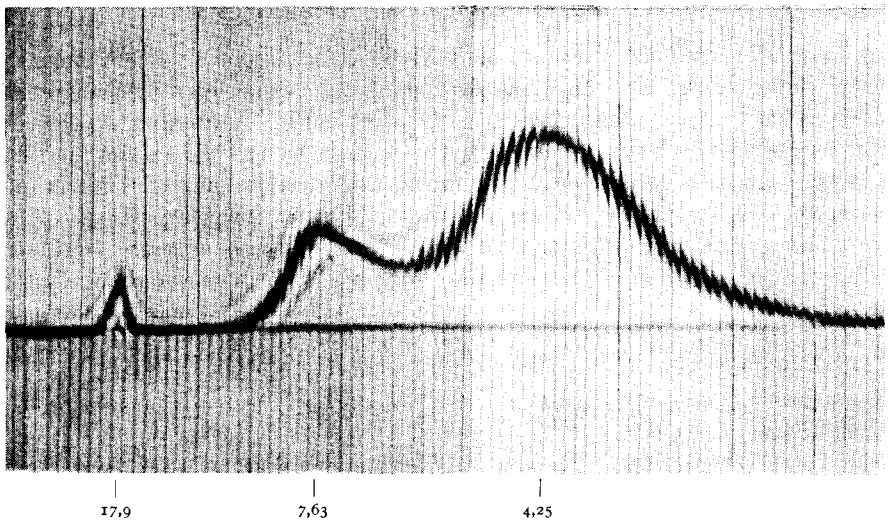

Fig. 1. B. Similar preparation photographed by method described in text. Bar angle 50°, 56,100 rev./min. Photographs taken at 2-min intervals.

this the time at operating speed producing an effect equal to that incurred during acceleration. Subtracting this time from the time at which the first picture was taken gave a value for zero time. When the distance from the meniscus to the peaks (measured on conventional photographs of the Schlieren diagram) was plotted against time, the lines obtained did not converge exactly at the meniscus level at zero time for the evident reason that a period of time is required to form the boundary at the meniscus. The method described is therefore subject to this error. Considerable improvement in accuracy could be obtained by using a synthetic boundary cell to form the boundaries and a continuous film drive combined with a very narrow slit to obtain a smoother curve.

In Fig. 1B the photographs taken through the slit are spaced evenly along a time scale. Since the less rapidly sedimenting proteins will pass the slit more slowly, their peaks will occupy a larger area than more rapidly sedimenting peaks produced by the same concentration of protein. The area under each peak will therefore not be proportional to the concentration of the constituents unless the area is divided by the elapsed time.

Since the chief use of this method will probably be illustrative, the sedimentation coefficients may be determined in separate experiments and used to calculate the values for the abscissa as has been done in Fig. 1B.

*Biology Division, Oak Ridge National Laboratory**, *Oak Ridge, Tenn. (U.S.A.)*

NORMAN G. ANDERSON
R. E. CANNING

1 G. L. MILLER AND R. H. GOLDER, *Arch. Biochem.*, 29 (1950) 420.

Received August 18th, 1959

* Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.