of Eq. (1), and this is illustrated by the three schematic curves of Fig. 1. The successive steps of curve

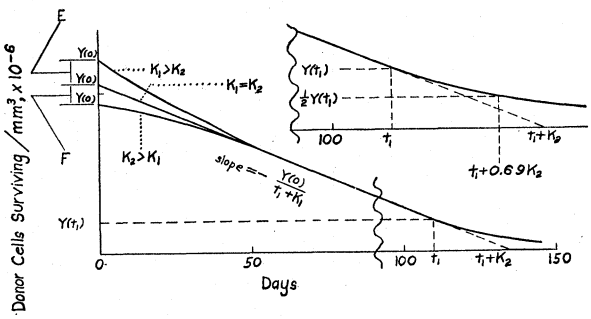

FIG. 1. Three normal schematic Ashby curves of donor red blood cells in the recipient, with magnification × 2 of the tail.

analysis are the determinations of (*a*) time t_1 (at which the curvature starts to increase); (*b*) intercept (t_1+K_2) (and hence K_2 by subtracting the t_1 of $[a]$); (*c*) the linear slope $-Y(0)/(t_1+K_1)$ (and hence K_1 by subtracting t_1 of $[a]$ from $Y(0)$ times the negative reciprocal of this slope); (*d*) the half-life on the tail $(t_1+0.69\,K_2)$ (and hence a check on K_2 by subtracting the t_1 of $[a]$); (*e*) the agreement between Y (t_1) and $\frac{K_2Y(0)}{(t_1+K_1)}$; and, if $K_1 \neq K_2$, (*f*) the agreement between E (or F) and $(K_1-K_2)Y(0)/(t_1+K_1)$. The last three items check the preceding determinations.

Typical experimental curves (*2–5*) yield results of 100–110 days for the wearing time t_1 and of 15–40 days for the average life of worn cells. These results of the present interpretation agree with the conclusions of earlier interpretations in which the curve was linear at the beginning and in which the intercept (t_1+K_2) was said to give the life span of the donor's red cells (since $K_1=K_2$ in these cases). It is to be noted that the survival time of the donor cells, *in the donor,* is $Y(0)$ times the negative reciprocal of the slope of the linear portion of the curve.

The present interpretation also explains why some of the normal curves are not linear near the beginning—in terms of the different rates of random breakdown in the donor and recipient environments (each of which is determined explicitly when $K_1 \neq K_2$).

In case the donor has sickle cell anemia (*6, 7*), congenital hemolytic jaundice (*8*), or acquired hemolytic anemia (*2, 5, 8*), the wearing time t_1 is negligible and the results are expressed by Eq. (2). This expression is linear when it is plotted on semilogarithmic paper because log Y is in this case a linear function of time. On measuring this semilogarithmic plot with a ruler, one finds that K_2 is the number of multiples of 2.30 days corresponding to each power of 10 through which Y changes.

References

1. ASHBY, W. *J. Exptl. Med.,* **29**, 267 (1919).
2. BROWN, G. M., *et al. J. Path. Bact.,* **56**, 81 (1944).
3. CALLENDER, S. T., POWELL, E. O., and WITTS, L. J. *Ibid.,* **57**, 129 (1945).
4. BERLIN, R. *Acta Med. Scand.,* Suppl., **139**, 1 (1951).
5. MOLLISON, P. L. *Clin. Sci.,* **6**, 137 (1947).
6. SINGER, K., *et al. J. Lab. Clin. Med.,* **33**, 975 (1948).
7. CALLENDER, S. T., *et al. Ibid.,* **34**, 90 (1949).
8. LOUTIT, J. F., and MOLLISON, P. L. *J. Path. Bact.,* **58**, 711 (1946).

Manuscript received November 1, 1951.

The Ultracentrifugation of Soluble Cytochromes[1]

W. W. Wainio, Bertram Eichel, and S. J. Cooperstein

Rutgers University, New Brunswick, New Jersey, Western Reserve University, Cleveland, Ohio, and The Marine Biological Laboratory, Woods Hole, Massachusetts

A particulate preparation of mammalian heart muscle dissolved with the aid of sodium desoxycholate has been studied in the ultracentrifuge.[2] The method of preparation and the visible absorption spectra have been previously described (*1*). The designation Preparation O-4 refers to the amount of sodium desoxycholate used to dissolve the proteins. As pointed out previously, there is evidence that the cytochromes *b, c,* and oxidase are present in this preparation.

A concentrated solution of the lyophilized Preparation O-4 was made by dissolving each "ml equivalent"[3] in 1 ml water. It was centrifuged at 260,000 × *g* (59,780 rpm) for 150 min. At this time, the preparation contained one slow-moving boundary. The ultracentrifuge was stopped, using the rapid brake, and the upper two thirds of the contents of the cell were removed for spectral analysis. The solution was transferred to a microcuvette (*2*) of the spectrophotometer,[4] reduced with $Na_2S_2O_4$, and covered with mineral oil for spectral analysis. The curve presented in Fig. 1 indicates that a small amount of cytochrome oxidase is present (the 603-mμ peak), but that the principal component is cytochrome *c* (the 550- and 520-mμ peaks). All the cytochrome *b* and most of the cytochrome oxidase have sedimented. Cytochrome *c,* therefore, is the most slowly sedimenting chromoprotein.

In another experiment the concentrated solution of the enzyme was reduced with $Na_2S_2O_4$ before being introduced into the ultracentrifuge cell. This was accomplished by filling the cell with the oxidized preparation (at which time the last bubble was removed) and by layering the reduced preparation on the bottom of the cell, thus forcing the oxidized preparation

[1] A preliminary centrifugation of a Preparation 2-3 (*1*) was done for us by Lawrence J. Milch, USAF, School of Aviation Medicine, Randolph AFB, San Antonio, Texas. We wish to thank Major Milch for his willing collaboration. This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

[2] We thank L. Robinson Hyde for assisting and instructing us in the use of the Spinco instrument.

[3] One "ml equivalent" represents the amount of dry preparation obtained by lyophilizing 1 ml of the original supernatant.

[4] The Beckman spectrophotometer was made available through the courtesy of Arnold Lazarow.

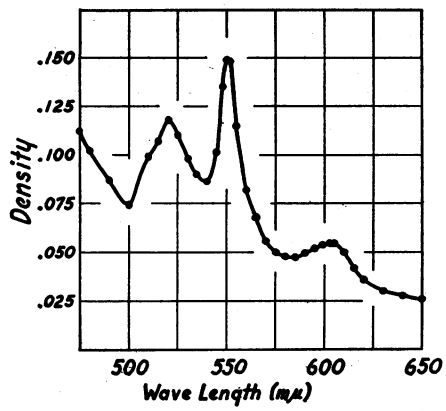

FIG. 1. Spectrum (reduced) of the upper two thirds of the contents of the ultracentrifuge cell after 150 min at 260,000 × *g*.

out at the top. After 70 min at 260,000 × *g* (59,780 rpm) the ultracentrifuge was stopped in the following way to minimize mixing of the contents: the rapid brake was applied to 30,000 rpm, the slow brake to 5,000 rpm, and then the head was allowed to coast until it had come to rest. A photograph taken at 30,000 rpm (74 min) represents approximately the position of the boundaries when the centrifuge had come to rest (Fig. 2). There are at least four components present. The head was detached, and the cell was cautiously removed and placed in a spectrophotometer carrier that had previously been adapted to this use.[5] The spectra were determined at four levels corresponding to positions 9½, 7, 4½, and 2 mm above the floor of the cell, as indicated approximately by the arrows above the numbers 1, 2, 3, and 4 in Fig. 2. The holes, each 1 mm in diameter, were drilled into a slide which was placed between the slit

[5] Details will be furnished on request.

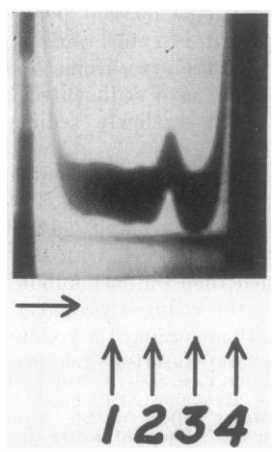

FIG. 2. Sedimentation pattern corresponding approximately to the relationships prevailing with the head at rest. Arrow 1 indicates approximately the position of the hole nearest the top of the cell when the spectra were taken. Time, 74 min; speed, 59,780 rpm (photo taken at 30,000 rpm); cell vol, 0.8 ml; temp, 22°; bar angle, 50°; exposure time, 20 sec; filter, Wratten #16; buffer, 0.1 *M* $Na_2HPO_4 \cdot KH_2PO_4$; pH, 7.4.

and the centrifuge cell. The four curves are presented in Fig. 3. Curve 1 represents the spectrum obtained through the hole nearest the top of the cell. There is little or no cytochrome *b* at this level, suggesting that this component has sedimented. A comparison of the curves in the order 1, 2, 3, and 4 shows that cytochrome *b* is nearer the bottom of the cell than is cytochrome oxidase. The peaks at 530 and 560 mμ (cytochrome *b*) increase as the bottom of the cell is approached and are prominent on Curve 4 in relation to the 603-mμ peak (cytochrome oxidase). It can be concluded that of these two chromoproteins the more rapidly sedimenting component is cytochrome *b*.

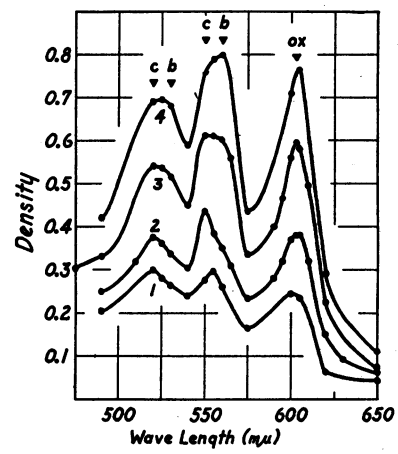

FIG. 3. Spectra at four levels in the ultracentrifuge cell of the reduced Preparation O-4 after 74 min at 260,000 × *g*. Curve 1 obtained through the hole nearest the top of the cell.

An inspection of the peak of cytochrome oxidase on the several curves of Fig. 3 shows a symmetry about 603 mμ. There is no evidence here for the separation of cytochrome oxidase into two components.

From a series of photographs taken of the sedimenting components, it has been calculated that the fastest moving boundary, probably representing cytochrome *b*, has an uncorrected sedimentation constant of 6.5×10^{-13} sec. The second and third boundaries have uncorrected constants of 5.8 and 5.2×10^{-13} sec, respectively. The slowest moving boundary, probably representing cytochrome *c*, has an uncorrected constant of 1.2×10^{-13} sec. This last value is to be compared to the published, corrected value of 1.9×10^{-13} sec obtained by Pedersen and Andersson (*3*) for cytochrome *c*.

In conclusion, it is to be noted that cytochrome *b* is the most rapidly sedimenting component, cytochrome oxidase is intermediate, and that cytochrome *c* sediments most slowly.

References

1. EICHEL, B., *et al. J. Biol. Chem.*, **183**, 89 (1950).
2. LOWRY, O. H., and BESSEY, O. A. *Ibid.*, **163**, 633 (1946).
3. PEDERSEN, K. O., and ANDERSSON, K. J. I. Quoted by H. P. Lundgren and W. H. Ward in D. M. Greenberg (Ed.), *Amino Acids and Proteins*. Springfield, Ill.: Thomas, 394 (1951).

Manuscript received December 3, 1951.