ribonucleic acid in the liver, we may picture the amount of energy available as the factor determining their rates of synthesis, provided no other component in the synthetic mechanism limits the response to a change in available energy.

Such a relationship is capable of explaining the findings of Campbell & Kosterlitz (1949) that the total turnover of ribonucleic acid and phospholipid in the liver of the rat increases during pregnancy, since, in the rat, energy intake exceeds requirements during pregnancy (Morrison, private communication), and on the above hypothesis this would be expected to lead to such a change in the rates of synthesis of ribonucleic acid and phospholipid.

SUMMARY

1. A study has been made of the effect of variations in energy intake on the amount of phospholipid in the rat liver and its uptake of labelled phosphorus.

2. When the diet provided an adequate supply of protein, the amount of phospholipid in the liver rose in response to increments in energy intake, whereas it failed to do so when the diet was devoid of protein.

3. The total turnover rate of phospholipid was influenced by energy intake only when the diet contained protein. It is suggested that the rate of phospholipid synthesis on protein-free diets is unaffected by energy intake within the range studied because some other component in synthesis is reduced to a level at which it becomes the factor determining the rate of synthesis.

4. The relationship of energy balance to the rates of synthesis of protein, phospholipid and ribonucleic acid is discussed.

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Sedimentation Coefficients of Small Molecules: Methods of Measurement Based on the Refractive-Index Gradient Curve. The Sedimentation Coefficient of Polyglucose A

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Svedberg & Pedersen (1940, p. 283) and Gutfreund & Ogston (1949) have proposed methods for measuring the sedimentation coefficient,* s , when a substance sediments too slowly for s to be measured in the usual way, from the movement of the maximum

* The term 'sedimentation coefficient' instead of 'sedimentation constant' has been introduced to indicate that it is not a constant except under specified conditions.

gradient of refractive index. In the case of slowly-sedimenting molecules, the boundary remains sufficiently close to the meniscus that the shape of the boundary curves must be predicted for the case of restricted rather than free diffusion. Boundary curves like those of Fig. 2 result, in which the maximum gradient of refractive index may remain at the meniscus throughout the centrifuge run.

Both the methods of Svedberg & Pedersen and Gutfreund & Ogston are suitable for use with boundary curves given in the form of concentration, or some quantity proportional to concentration against distance in the cell. The purpose of this paper is to compare these methods and to show how they may be simplified for use with boundary curves in the form of refractive index gradient against distance in the cell.

Fig. 1. Representative graph of concentration against distance in the cell. (i) The initial uniform concentration is c_0 ; at time t it becomes $c_0 e^{-2s\omega^2 t}$ or c_t (cf. Svedberg & Pedersen, 1940, p. 7). (ii) The origin for the x axis is the centre of rotation. (iii) x_0 is the meniscus. (iv) x_b marks the position of 'the infinitely sharp boundary': $x_0 e^{s\omega^2 t}$ (Svedberg & Pedersen, 1940, p. 18). (v) X is a reference plane in 'the constant part of the cell' where $dc/dx=0$ and the concentration is c_t . (vi) The area of a plane at x is kx , since the cell is a truncated wedge whose two non-parallel sides would converge at the centre of rotation.

THEORY

The derivation of Gutfreund & Ogston rests on a statement of the conservation of mass. Using their symbols, explained in Fig. 1, the amount of material between X and x_0 before sedimentation starts ($\frac{1}{2}kc_0(X^2 - x_0^2)$) is equated to the amount remaining at time t ($\int_{x_0}^X kcx dx$) plus the amount which has sedimented past X ($\int_0^t J_X dt$), where J_X is the transfer in unit time of material past X ; writing J as concentration times area times velocity gives

$$\int_0^t c k X s \omega^2 X dt = \frac{1}{2} k c_0 X^2 (1 - e^{-2s\omega^2 t})$$

$$\frac{1}{2} k c_0 (X^2 - x_0^2) = \int_{x_0}^X k c x dx + \frac{1}{2} k c_0 X^2 (1 - e^{-2s\omega^2 t}) \quad (1)$$

It is important to notice that s appears in equation (1) only as the sedimentation rate in the constant part of the cell. Thus the sedimentation coefficient, unlike a measured diffusion coefficient, refers to one definite concentration. In general the sedimentation coefficient depends on the concentration and it might be thought that, since s is measured from the boundary curves, it would correspond to some concentration within the boundary. However,

when s is measured by this or any corresponding equation, the derivation above shows that the concentration to which it refers is that of the constant part of the cell. Because of the sector-shaped cell and changing field strength, this concentration slowly decreases with time ($c_t = c_0 e^{-2s\omega^2 t}$). When the substance is heterogeneous, the use of equation (1) gives a weight-average sedimentation coefficient which is slightly time-dependent, because of the dilution effect mentioned above (see Kinell, 1947). In a system such as insulin, where interacting components are present, the constituent sedimentation coefficient (cf. Svedberg & Pedersen, 1940, p. 28) is obtained.

The form of equation (1) obtained by Gutfreund & Ogston (1949) is inconvenient for use with the optical systems now generally employed, for example the Lamm scale method (Svedberg & Pedersen, 1940, p. 254), the Philpot schlieren system (Philpot, 1938), and their variants, because of the time-consuming numerical integration required, although it would be well-suited to the light absorption (Svedberg & Pedersen, 1940, p. 240) or the integral fringe interferometric systems (Philpot & Cook, 1948).

This difficulty can be overcome by integrating the expression for the amount remaining between X and x_0 by parts

$$\left(\text{this becomes } \frac{1}{2} k c_t X^2 - \frac{1}{2} k c_{x_0} x_0^2 - \frac{1}{2} k \int_{x_0}^X x^2 \frac{dc}{dx} dx \right).$$

The equation stating the conservation of mass can now be written as

$$\frac{1}{2} k c_0 (X^2 - x_0^2) = \frac{1}{2} k c_0 X^2 (1 - e^{-2s\omega^2 t}) + \frac{1}{2} k c_t X^2 - \frac{1}{2} k c_{x_0} x_0^2 - \frac{1}{2} k \int_{x_0}^X x^2 \frac{dc}{dx} dx. \quad (2)$$

This equation can be simplified by replacing c_{x_0} by $(c_0 e^{-2s\omega^2 t} - \int_{x_0}^X \frac{dc}{dx} dx)$ and remembering that $c_t = c_0 e^{-2s\omega^2 t}$:

$$c_0 x_0^2 = x_0^2 \left(c_0 e^{-2s\omega^2 t} \int_{x_0}^X \frac{dc}{dx} dx \right) + \int_{x_0}^X x^2 \frac{dc}{dx} dx. \quad (3)$$

It is convenient, for the purpose of calculation, to replace $\int_{x_0}^X x^2 \frac{dc}{dx} dx$ by

$$\int_{x_0}^X (x - x_0)^2 \frac{dc}{dx} dx + 2x_0 \int_{x_0}^X (x - x_0) \frac{dc}{dx} dx + x_0^2 \int_{x_0}^X \frac{dc}{dx} dx.$$

Solving for s , after making this substitution, gives

$$-\frac{2s\omega^2 t}{2.303} = \log_{10} \left\{ 1 - \left(\left[\int_{x_0}^X (x - x_0)^2 \frac{dc}{dx} dx + 2x_0 \int_{x_0}^X (x - x_0) \frac{dc}{dx} dx \right] / c_0 x_0^2 \right) \right\}. \quad (4)$$

This equation can be used directly and simply for the calculation of s . As long as the refractive-index increment is directly proportional to the concentration, dc/dx can be replaced by dn/dx , the refractive index gradient, and c_0 by Δn , the difference in refractive index between the solvent and the original solution.

No formal derivation is given by Svedberg & Pedersen, although measurements of s by their method have been reported by Pedersen & Li (1950). Their procedure for calculating s (Svedberg & Pedersen, 1940, p. 283) is equivalent to the following equation:

$$\int_{x_0}^x c dx = c_t(X - x_b), \quad (5)$$

where $x_b = x_0 e^{s\omega^2 t}$, the position that the boundary would have if it were infinitely sharp. Integrating by parts once more and rearranging gives a form more suitable for use with refractive-index gradient curves

$$x_b - x_0 = \frac{\int_{x_0}^x (x - x_0) \frac{dc}{dx} dx}{c_t}. \quad (6)$$

If the boundary gradient curve is symmetrical, the first moment (given by the right-hand side of equation (6)) coincides with the centre of the boundary; this is the justification for the usual practice of measuring s from the rate of movement of the maximum gradient of refractive index.

A disadvantage of equation (6) for measuring s , as compared to (4), is that a preliminary estimate of s is needed in order to find c_t , since $c_t = c_0 e^{-2s\omega^2 t}$, and $x_b - x_0$, from which s is computed. The condition on the provisional value, s' , for obtaining s with an accuracy of 1%, is that $2\omega^2 t |s - s'| < 0.01$, where t is the time of the final photograph. Since this time depends upon the rate at which the boundary spreads as well as upon s , it is not possible to make a more specific statement of the allowable error in $|s - s'|$. For the experiments reported here, $\omega^2 t$ did not exceed $2.5 \times 10^{11} \text{ sec.}^{-1}$ and a $|s - s'|$ value of $0.2s$ would have caused an error of 1% in s .

Equations (5) and (6), derived from the procedure of Svedberg & Pedersen, are not consistent with the equation derived by Goldberg (1953). He has found that x_b is given by the square root of the second moment of the gradient curve (taking the centre of rotation as the origin for this moment) rather than the first moment specified by equation (6). His equation agrees with that previously derived by Gutfreund & Ogston. The error in using the first moment, rather than the square root of the second, is very small; in usual cases this error is about 0.2% and it would rarely exceed 0.5%.

MEASUREMENTS AND CALCULATIONS

The only special quantities required for the measurement of s by these methods are

$$\Delta n, \quad \int_{x_0}^x (x - x_0)^2 \frac{dn}{dx} dx \quad \text{and} \quad \int_{x_0}^x (x - x_0) \frac{dn}{dx} dx$$

for equation (4) and, for equation (6),

$$\Delta n e^{-2s\omega^2 t} \quad \text{and} \quad \int_{x_0}^x (x - x_0) \frac{dn}{dx} dx.$$

Δn may be calculated from the concentration when the specific refractive increment is known or, preferably, may be measured directly by a differential refractometer. When the constants of the optical system are known the schlieren photographs may be converted directly into graphs of dn/dx versus x (cf. Cecil & Ogston, 1948; Svedberg & Pedersen, 1940, p. 259). The theoretical uncertainty in this procedure is roughly equal to the precision of measurement (cf. Adler & Blanchard, 1949). Corresponding base-lines must be obtained from centrifuge runs with buffer only.

The integrals, or moments, listed above are found by numerical integration: values of dn/dx —about fifteen in number—are taken at a constant interval, Δx , throughout the boundary and the sums

$$\Delta x \sum_i (x_i - x_0) \left(\frac{dn}{dx} \right)_i \quad \text{and} \quad \Delta x \sum_i (x_i - x_0)^2 \left(\frac{dn}{dx} \right)_i$$

are then computed. This procedure, which is equivalent to replacing the definite integral by a set of trapezoids, introduces an uncertainty of less than 1% into the value for these integrals. With the use of a modern calculating machine these two sums may be computed simultaneously; this takes about half-an-hour for a series of six or eight photographs. The most time-consuming step in calculating s by these methods is measuring the values of dn/dx against x .

If the speed and temperature vary during the centrifuge run, $-\frac{2s\omega^2 t}{2.303}$ should be replaced, in equation (3), by

$$-\frac{2S_{20}}{2.303} \int_0^t \frac{\eta_{20}}{\eta_T} \omega^2 dt;$$

this integral is easily evaluated by trapezoidal integration.

S_{20} is then found from the slope of the plot $\int_0^t \frac{\eta_{20}}{\eta_T} \omega^2 dt$ against the right-hand side of equation (3). The values in Table 1 were obtained by the method of least squares. This allows one to estimate the standard deviation of the measurement as well as giving the best estimate for s (cf. Mather, 1943, p. 115). When equation (5) is used, S_{20} can be found from the slope of the plot suggested by Cecil & Ogston (1948) or from the plot of

$$2.303 \log_{10} \frac{x_b}{x_0} \quad \text{versus} \quad \int_0^t \frac{\eta_{20}}{\eta_T} \omega^2 dt;$$

the former is preferable when there is any leakage from the cell.

EXPERIMENTAL

The synthetic polyglucose used for these sedimentation studies was prepared by Dr P. W. Kent. Its preparation and properties have recently been described (Kent, 1953). The procedure for the centrifuge runs was that of Cecil & Ogston (1948). A layer of liquid paraffin was used to seal the cell

and prevent evaporation. The speed of rotation was 1000 rev./sec.; it was measured by an apparatus counting the revolutions of the rotor, designed and built by Dr R. Cecil, which gives the mean speed with an accuracy of 0.01%.

The edge-schlieren system has been replaced by a bar-schlieren system (cf. Fig. 2) in order to improve the precision of measuring dn/dx values. The bar-schlieren system used

Fig. 2. Schlieren photographs showing the sedimentation of polyglucose A at concentration 1.40% (w/v). Each photograph represents a graph of refractive-index gradient versus distance in the cell. The fine vertical line near the right-hand side of each picture represents the meniscus between liquid paraffin and polyglucose solution. The time between photographs was 10 min., the speed of rotation 1000 rev./sec. The bar angle was 14.5° for the first four pictures and 18.5° for the last two.

differs from that described by Kegeles & Gutter (1951) in minimizing, rather than emphasizing, the effects of diffraction at the bar; a relatively narrow bar (0.5 mm.) and wide horizontal source slit (0.5–2.0 mm., depending upon the bar angle) are used. The bar-schlieren system has an advantage over the corresponding slit system in that sharpness of the schlieren diagrams increases with exposure time. A two-way travelling microscope, aligned parallel with the meniscus trace, was used to measure the photographic plates; the reproducibility of the maximum dn/dx values is $\pm 1\%$ for this range of concentration.

Reference base-lines were obtained from a run using the same cell but containing only solvent, 0.1 M-NaCl. The shape of the base-line, at a given time, speed and bar angle, was assumed to be the same in the reference run and in the run containing solute or 'solution run'. The effect, discussed by Cecil & Ogston (1948), of the change in refractive index across the boundary is not sufficiently large that it has to be taken into account. The position of the base-line was found by superimposing a schlieren photograph from the reference run on to the corresponding one from the solution run, in the constant part of the cell.

Bulging of the cell at high speed causes material to be trapped in the bottom and it is often necessary to wash this out in order to obtain corresponding base-lines from one run to the next. The cell is refilled after a centrifuge run with water and a small amount of CCl_4 , and the centrifuge is then taken back up to full speed. The CCl_4 displaces any solution from the preceding run caught in the bottom of the cell; any CCl_4 similarly trapped is easily removed by evaporation.

The constants of the optical system have been redetermined and agree with previous values (Cecil & Ogston, 1948); given Δn it is possible to calculate the area under a resolved schlieren peak with an uncertainty of less than 1%. Δn was measured with the differential refractometer described by Cecil & Ogston (1951), which now has a one-slit diffraction pattern in place of the two-slit interference pattern formerly used to measure the deflexions.

The correction for the rotor thermocouple has been re-determined by the procedure of Cecil & Ogston (1948), from several centrifuge runs with diphenyl ether. This correction is considered to be accurate within $\pm 0.2^\circ$.

These centrifuge runs must be continued for long periods of time in order to obtain steady-state readings; one lasted more than 13 hr. It was found necessary to reject readings unless *all* variables (thermocouple readings, depth of liquid diphenyl ether, rotor speed and turbine oil pressure) were constant for more than 30 min. Of these, constant position of the diphenyl ether meniscus is particularly important.

DISCUSSION

Sedimentation behaviour of polyglucose A

The sedimentation coefficient of polyglucose A does not show a measurable dependence on concentration in the range studied (see Table 1). However, the accuracy of measuring s by these methods, compared with that of finding the centre of a symmetrical boundary, decreases rather rapidly with decreasing concentration because the accuracy of measuring dn/dx values depends upon their magnitude and consequently upon the total concentration.

Table 1. *Variation of sedimentation coefficient (in Svedberg units) with concentration of polyglucose A*

((a) Obtained by using equation (4). (b) Obtained by using equation (6). (c) Average of (a) and (b), corrected to the viscosity of water at 20° and also corrected for the difference in density of water and 0.1M-NaCl, assuming a partial specific volume of 0.62 for the polyglucose. The temperature of the centrifuge runs was 28°. The measurement of s in each case was based on six schlieren photographs taken at 10 min. intervals.)

c (%, w/v)	(a) $s_{20} \pm \text{S.D.}$	(b) $s_{20} \pm \text{S.D.}$	(c) s_{20}, w
1.40	0.820 ± 0.020	0.800 ± 0.018	0.82
0.97	0.774 ± 0.019	0.769 ± 0.019	0.78
0.61	0.789 ± 0.031	0.783 ± 0.030	0.80

It is not possible to say how homogeneous the polyglucose is from the sedimentation data because the theory for determining heterogeneity from the rate at which the boundary spreads with time (Williams, Baldwin, Saunders & Squire, 1952) applies only to the case when the boundary resolves rapidly from the meniscus.

Comparison of results from equations (4) and (6)

The two methods of calculating s give the same value for s , within the standard deviation of the measurement, and also the same value for the standard deviation (cf. Table 1). The standard deviations reported here are less than half, in proportion to the corresponding values for s , of those reported by Gutfreund & Ogston (1949). Pedersen & Li (1950) do not give this datum. The limiting source of error in measuring s by these methods is probably in establishing the base-lines. The use of a double cell, as suggested by Philpot & Cook (1948), would undoubtedly improve their accuracy.

Use of these equations for other small molecules

It is interesting to note that the values for sucrose (0.25S) and potassium chloride (0.34S) predicted,

for infinite dilution, from molecular weight, diffusion coefficient and partial specific volume (cf. Svedberg & Pedersen, 1940, p. 5) are in a range suitable for study by these methods. However, diffusion is so rapid that the constant part of the cell disappears after a comparatively short time and these methods are not applicable after that time.

Kegeles (1952) and Pickels, Harrington & Schachman (1952) have described ultracentrifuge cells which may be used to form boundaries between solvent and solution. The boundary is formed far enough away from the meniscus that the boundary shape is characteristic of free diffusion and the movement of the maximum gradient of refractive index may be used to measure s . This technique should have several advantages over the methods described here for the measurement of sedimentation coefficients of small molecules.

SUMMARY

1. The methods of Svedberg & Pedersen (1940) and of Gutfreund & Ogston (1949) for calculating sedimentation coefficients of small molecules have been simplified for use with refractive-index gradient curves. A comparison of the two methods is presented.

2. The average sedimentation coefficient of polyglucose A (Kent, 1953) from measurements at three concentrations, is 0.80 Svedberg units. No dependence upon concentration was observed in this concentration range. The standard deviation of each measurement was about 3 %.

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