shoots supports this hypothesis. Our data have shown that the vitamin C concentration in the vegetative buds increased markedly just before opening, the peak occurring in very young leaves. In the reproductive buds and catkins there was a similar increase just before rapid growth began. In the phloem of the stem, adjoining the cambium, there was a peak concentration at the time of active growth. Schopfer (1943) has suggested that vitamin C may also be connected with photosynthesis and the production of sugars. Our data support this suggestion in so far as high concentrations of the vitamin were found in the mesophyll and in other green tissues (e.g. cortex of green stems) where photosynthesis was most marked. However, photosynthesis cannot very well explain the function of the vitamin C in the endocarp, where the highest concentrations occur. Since these concentrations fall rapidly when the woody shell is developing, and since in a given nut the concentration of vitamin C in the woody part is much lower than in the non-woody part, we suggest that the vitamin may be utilized in the formation of the lignin. This hypothesis may also explain the function of the vitamin C in the stem. The concentration gradient of vitamin C in the stem declines towards the roots. In the large trees yielding our main sample we could only follow this gradient 2 or 3 ft. down the shoots into the main stem. By examining a sapling about 4 ft. high we

were able to establish the complete gradient from the buds down to the finest roots (Table 6).

Table 6. *Concentration gradient of vitamin C in walnut sapling examined March 1945*

(C.Y.G. denotes current year's growth; L.Y.G., last year's growth.)		Vitamin C (mg./100 g.)
Buds		258
Stem cortex and phloem:	C.Y.G.	252
	L.Y.G.	183
Roots		140
Fine roots		94

SUMMARY

1. Studies on samples of different tissues collected during three seasons from *Juglans regia* trees growing in several districts round London showed, in the endocarp, remarkably high concentrations of vitamin C, which may be used in lignification. This vitamin C probably originates mainly in photosynthetic centres in the leaves.

2. Examination of the intervening tissues showed that physiological conditions (acidity, concentration of enzymes and of vitamin C) militate against translocation of vitamin C as such from leaf to nut. The experimental findings favour an alternative hypothesis, which will be considered later.

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The Accuracy of the Svedberg Oil-turbine Ultracentrifuge

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1. THE SEDIMENTATION CONSTANT

The values of sedimentation constants which appear in the literature show more variation than one would expect to obtain from measurements made on well defined substances by an accurate physical method. Measurements made in Oxford on several proteins which are believed to be definite and homogeneous have always given values lower than those obtained

elsewhere (Table 1); correction of these values for errors described in the present work would further increase these discrepancies. The large difference between the values for the sedimentation constant of insulin of Gutfreund & Ogston (1946) and of Miller & Andersson (1942) finally led us to investigate the errors of our Svedberg ultracentrifuge.

Table 1. *Comparison of sedimentation constants from various sources*

Substance	Author	Concn. (g./100 ml.)	S_{20} (corr.) $\times 10^{13}$
Horse serum, albumin (crystallized, carbohydrate-free)	Kekwick (1938) (Uppsala)	—	4.48
	(Oxford, unpublished)	1	4.31
Ovalbumin	Svedberg & Pedersen (1940)	0.5	3.55
	(Oxford, unpublished)	1	3.4
Insulin	Miller & Andersson (1942)	0	3.55
	Gutfreund & Ogston (1946)	0.1	3.34
Lactoglobulin	Pedersen (1936)	0.1	3.12
	Johnston & Ogston (1946)	1	2.83
	This work	0	2.83

Provided that sedimentation is undisturbed by mechanical vibration or by thermal convection, the accuracy with which a sedimentation constant can be determined should be limited only by the accuracy of the measurement of the following quantities: (1) the positions of the sedimentation boundary at stated times, relative to the meniscus and to the centre of rotation; (2) the speed of rotation; (3) the state of the solution with respect to composition and temperature.

Since no substance is available whose sedimentation constant is certainly known, it was necessary to test for sources of inaccuracy piecemeal. Lactoglobulin was used as a working substance, since it appears to be one of the best defined and most stable proteins.

The accuracy of the measurement of the positions of the boundary and of the speed of rotation were easily checked; the chemical composition of the solution was a matter of ordinary technique; the question of cell temperature proved more complex, and, before attempting to measure it, we had to examine the factors which may affect it.

Calculation of the sedimentation constant

The traditional method of calculation is to obtain an estimate of the 'uncorrected' sedimentation constant S_{20} from the movement of the boundary during each of a succession of intervals; S_{20} is the rate of movement, reduced to unit gravitational field and corrected for the effect of temperature on the viscosity of the solvent. The effects of temperature on the density of the solvent, and of the buffer salts on its density and viscosity, do not vary appreciably during the course of a run and are, therefore, applied as corrections to the mean value $\bar{S}_{20}$, to give $\bar{S}_{20}$ (corr.); this is the rate of movement in water at 20° under unit gravitational field.

This procedure of averaging the estimates of S_{20} obtained in successive intervals does not, however, make nearly full statistical use of the data available. A method was therefore devised which does so.

The value of S_{20} from a given interval is given by

$$S_{20} = \frac{\Delta x}{\Delta t} \frac{\eta_0}{\eta_{20}} \frac{1}{(A - \bar{I} + \bar{x}) \omega^2}, \quad (1)$$

where Δx is the distance moved during time Δt ; η_0 , η_{20} are the viscosities of water at 0° and 20°; A is the distance of the index from the centre of rotation; $\bar{I}$ the mean distance of the meniscus from the index and $\bar{x}$ the mean distance of the boundary from the meniscus, so that $(A - \bar{I} + \bar{x})$ is the mean distance of the boundary from the centre of rotation during that interval; ω is the mean angular speed of rotation. Since S_{20} is appreciably constant, and since an error of x contributes only a very small proportionate error to $(A - \bar{I} + \bar{x})$, equation (1) may be rearranged and integrated, without sensible error, to a linear equation in x :

$$x = S (\Delta x) = S_{20} S [\Delta t \eta_{20} / \eta_0 (A - \bar{I} + \bar{x}) \omega^2] = S_{20} S (\Delta t'), \quad (2)$$

S_{20} is then the slope of a plot of x against $S (\Delta t')$ and its value, with its standard deviation, may be calculated by the standard method of obtaining linear regression coefficients. Corrections for the effects of temperature on density and of the buffer salts are then applied as usual.

Using this method, good linear plots were obtained (Fig. 1); the standard deviation of the estimate of S_{20} seldom exceeded 0.5 % (Table 2).

EXPERIMENTAL

Optical system and measurement

The optical system was the original Philpot (1938) diagonal-schlieren system, modified by replacing the fused silica windows of the rotor casing and the compound quartz-fluorite lenses by a pair of simple glass lenses. A considerable improvement of definition resulted from this replacement. The source of light was a 250 W. Mazda box-type mercury lamp used with a monochromator; this reduced exposures tenfold compared with those previously required, and caused a further sharpening of the image, since the boundary did not now move an appreciable distance during an exposure (4–10 sec.).

The magnification of the spherical (schlieren) lens was measured over the whole field by photographing a finely ruled scale or its projected image, with the rotor first static and then running in the evacuated casing; the same value was obtained in each case, constant to 0.1% over the field.

Fig. 1. Plot of a run by equation (2). Ordinate: $S(\Delta x)$ in cm. measured on the photographic record; abscissa: $S(\Delta t')$ in cm. measured on the photographic record $\times 10^{-11}/\text{sec.}$

Measurement of the plates was made by means of a travelling microscope reading correct to 0.001 cm. The position of the boundary was taken as the maximum of the 'peak' in the diagonal-schlieren diagram, and was obtained to 0.005 cm. or less, according to the thickness of the boundary.

The distance of the index hole in the balance cell from the centre of rotation was measured to 0.05%.

The speed of rotation

The Hartmann Kempf reed frequency meter, for measuring the frequency of the alternating current generated by the rotor, was supplemented with a frequency bridge designed and built for us by Mr R. P. Martin and Mr P. A. Davenport, of the Clarendon Laboratory (to be published). This enables the speed to be measured to 0.1% in 4 frequency ranges, 300 ± 5 , 450 ± 5 , 700 ± 10 and 1000 ± 10 revs./sec. The bridge reads continuously within these ranges, so that control of speed drift is made easy. The bridge was calibrated at intervals against a crystal clock. During a run, the speed was kept constant within ± 5 revs./sec. and rarely changed by more than 2 revs./sec. during any 10 min. interval between photographs. All alterations of the turbine oil pressure were made at the beginnings of intervals, so that the drift of speed during any interval was nearly constant. The use of the bridge effected a noticeable reduction of the standard deviation of S_{20} , compared with estimates from runs in which the speed was measured by the Hartmann-Kempf meter.

The cell temperature

The rotor runs, on plain journal bearings, in a casing which contains hydrogen at low pressure. Heat is generated in the bearings and by friction with the hydrogen; it is lost to the cooled turbine oil and to the casing; the casing loses heat to the turbine oil and to the surrounding air. The temperature of the rotor, and thence of the cell contents, is thus controlled by several factors; it is measured by means of a thermocouple whose junction has a clearance of 0.025 cm. on the rotor surface. Svedberg & Pedersen (1940) state that this thermocouple reads the cell temperature within 0.5° . However, a change in the pressure of turbine oil, or of the pressure of hydrogen, causes so rapid a change in the reading of the thermocouple as to make it unlikely that this corresponds directly with a change of temperature of the whole mass of the rotor. It seems likely, on the contrary, that the temperature of the thermocouple, although controlled by the same factors as control that of the rotor, is not simply related to it.

Since it would be laborious to calibrate the thermocouple in terms of cell temperature, for variation of all possible factors, our aim was to find standard conditions which could be easily reproduced and then to calibrate under these conditions. A series of runs was, therefore, carried out, using a solution of lactoglobulin (1 g./100 ml.) in buffer (0.1 M-NaCl, 0.1 M-Na acetate, 0.04 M-acetic acid), to test the effects of varying conditions on the apparent sedimentation constant. The results were as follows (Table 2):

(a) In our former procedure, the cold water which cools the turbine oil was turned on at the beginning of the run: the temperature of the rotor thermocouple rose rapidly during speeding up and then, when full speed was reached, drifted upwards steadily for several hours ($0.1\text{--}0.3^\circ/10$ min.). When the cooling water was not turned on until full speed had nearly been reached (20–25 min. after starting), the temperature rose more rapidly at first, but reached a steady value within 50–60 min. after starting (30 min. after full speed); thereafter it did not change by more than 0.2° . The apparent sedimentation constants obtained by the latter procedure were significantly lower (by 1–2%) than by the former (Table 2). The new procedure was adopted as standard.

(b) For some years we have used 5 mm. pressure of hydrogen in the casing, instead of the 20 mm. recommended by Svedberg & Pedersen (1940), because Philpot (personal communication) had observed that there was less risk of thermal convection at the lower pressure in experiments using acetone as solvent. The quick effect of a change of hydrogen pressure on the thermocouple reading led us to test the effect of hydrogen pressure on sedimentation constant. This was found to be considerable, higher values being obtained at pressures less than 10 mm. (Fig. 2 and Table 2). No significant change accompanied a further raising of the pressure to 20 mm.; we, therefore, adopted 10–12 mm. as standard.

(c) Other variations did not appear to exert any considerable effect. The average speed of rotation was always within a few cycles of 1000 rev./sec.; the room temperature varied between 19 and 22° in various runs, but there appeared to be no correlation between this and the apparent sedimentation constant; the degree of filling of the cell, the presence or absence of a layer of paraffin (lidded cells were used) and the cell thickness did not affect the result.

Table 2. S_{20} under various conditions by equation (2)

Run	Conditions				Apparent S_{20} (corr.) $\times 10^{13}$	True S_{20} (corr.) $\times 10^{13}$	s.d. of S_{20} (corr.) $\times 10^{13}$
	H ₂ pressure (mm.)	Rotor temp.	Paraffin present	Cell (mm.)			
	Experiments under standard conditions						
563	10	Steady	No	12	2.876	2.813	0.007
571	10	"	"	12	2.866	2.803	0.006
559	10	"	"	12	2.899	2.835	0.009
572	10	"	"	12	2.854	2.791	0.015
574	10	"	"	12	2.858	2.795	0.012
593	10	"	"	3	2.863	2.800	0.014
532	18	"	"	12	2.873	2.810	0.006
538	20	"	Yes	12	2.871	2.808	0.008
580	20	"	No	12	2.891	2.827	0.006
					Mean of S_{20} (corr.)	2.809	
					s.d. of mean		0.014
Variation of conditions							
558	10	Rising	No	12	2.934	—	0.009
577	10	"	"	12	2.930	—	0.010
527	5	Steady	"	12	2.917	—	0.008
530	5	"	"	12	2.929	—	0.011
578	5	"	"	12	2.927	—	0.009
533	c. 1	Rising	"	12	3.042	—	0.008
536	1	"	Yes	12	2.944	—	0.016
564	1	Steady	No	12	2.959	—	0.004

(d) A number of runs done under these standard conditions (Table 2) shows a standard deviation of the estimates of S_{20} (corr.) of 0.014 or 0.5%, which is little greater than the normal s.d. of the estimate from a single run. This indicates that there is no important cause of random variation operating between runs other than those operating within a given run, and thus further confirms the unimportance of such factors as mean rotor speed (within the narrow range used), cell filling and room temperature.

Fig. 2. Apparent S_{20} (corr.) $\times 10^{13}$ against the pressure of hydrogen in mm. of mercury; open circles, steady temperature; filled circles, rising temperature.

(e) A calibration of the reading of the rotor thermocouple could now be undertaken. First it was shown, by comparison with a National Physical Laboratory standard thermometer, to read its own temperature correct to 0.1°. Next calibration was carried out by the method given by

Fig. 3. Record of a melting point run. Open circles represent values obtained during upward drift of temperature; filled circles represent readings at thermal steady states.

Fig. 4. Record of steady-state points in two melting point runs. Abscissa: proportional to the pressure at the solid-liquid meniscus. Values obtained at different thermal steady states.

Svedberg & Pedersen (1940). Diphenyl ether was five times recrystallized and then melted at 26.7–26.9°. A carefully cleaned 3 mm. cell was filled with the melted substance, which was allowed to solidify before insertion of the cell in the rotor. After full speed was attained, a slow upward drift of the rotor temperature was allowed to take place (0.2°/10 min.). When melting had begun, photographic records of the positions of the solid-liquid meniscus and readings of the rotor thermocouple were taken at intervals. In order to imitate our standard conditions of thermal-steady state, three such states, at different temperatures, were attained in the course of two runs. Fig. 3 shows the depths of the meniscus plotted against the thermocouple readings: it is seen that the points obtained during a drift of temperature lie 0.2–0.3° above the line drawn through the steady state points. Fig. 4 shows the thermocouple readings for the three steady states plotted against numbers proportional to the pressures at the solid-liquid meniscus (the actual pressures cannot be given, since the density of the liquid was not accurately known); extrapolation to zero pressure (effectively equal to 1 atm.) gives 25.8°, 1.0° below the mean melting point. (Diphenyl ether removed from the cell after these runs showed an unchanged melting point.) This means that the apparent sedimentation constants obtained under these conditions must be multiplied by 0.978 to give the true value.

Lactoglobulin solutions

Lactoglobulin was made from fresh milk by the method of Palmer (1934). It was twice recrystallized and stored as crystals in water under toluene in the cold room; recrystallization was effected by dissolving the crystals in NaCl

solution, removing any insoluble material, adjusting the pH to 5.2 by dialysis against acetate buffer and dialyzing against water. Solutions were made by dissolving the crystals in buffer (0.1M-NaCl, 0.1M-Na acetate, 0.04M-acetic acid) and dialyzing against buffer. The concentration of protein was estimated refractometrically against diffusate, using 0.00180 as the specific refractive increment; the solution was then diluted with diffusate to give a concentration of 1 g. protein/100 ml. Solutions were kept in the cold room and did not change their sedimentation properties during storage.

DISCUSSION

This work shows that the Svedberg oil-turbine ultracentrifuge can give highly reproducible results provided that the conditions of running are carefully controlled, and that proper attention is given to the measurement of all the quantities concerned. This conclusion may hold good for other types of ultracentrifuge. The reproducibility which we claim compares favourably with other published data.

We believe, also, that we have controlled all important sources of systematic error, and that the results which we now quote are accurate to 0.5 % (s.d.). Our value of 2.81×10^{-13} for the sedimentation constant of lactoglobulin differs considerably from Pedersen's (1936) value of 3.12×10^{-13} . Further discussion of this discrepancy will be left to a later paper.

2. ESTIMATION OF THE QUANTITY OF A SEDIMENTING COMPONENT

Subject to the limitations found by Johnston & Ogston (1946), the amount of a sedimenting component, in terms of its refractive increment, may be estimated by measuring the area of the 'peak' of the diagonal-schlieren diagram due to this component. Comparison of this with the total refractive increment of the solution due to protein (measured against diffusate) gives information about the homogeneity of the preparation. A deficiency may be due to another visible component or to material which sediments too slowly or too quickly to be observed, or which is too heterogeneous to form a visible boundary. A single homogeneous component should give, by integration of the peak, a value equal to the total refractive increment.

The accuracy of measurement of the quantity of a component by the 'scale' method of Lamm (1934) appears to be of the order of 1 to 2 %; that of the diagonal-schlieren method of Philpot (1938) has been believed to be rather lower. We have modified the method of estimation of Philpot's method and now find its accuracy to be nearly equal to that of Lamm.

EXPERIMENTAL

Optical system

Modifications to the optical system described above have improved the photographic definition. The use of a high intensity mercury lamp as a source of monochromatic light has resulted in a reduction of exposure times by tenfold; as a result, the boundary does not move an appreciable fraction of its thickness during an exposure, and blurring of the limbs of the 'peak' is avoided. The line of demarcation on the photographic record (Fig. 5) is not quite sharp, but shows a narrow region of shading, from black to clear, due to diffraction; however, any line of constant photographic blackening is equivalent to the theoretical line of demarcation, being merely displaced by a constant distance at right angles to the cylindrical axis of the cylindrical lens. The width of the region of shading can be narrowed by making a high-contrast print from the original record; we find, however, that this procedure gives no noticeable increase in accuracy, except where the region of shading is broadened by use of the schlieren system at high optical sensitivity.

Following Johnston & Ogston (1946), we have made tracings on white paper of enlarged projections of the photographic records, using a linear magnification of 4.5. A line of constant blackening can be traced without difficulty.

Area measurements of sedimentation diagrams

The area to be measured is that included between the 'peak' due to the boundary and the base line from which this arises; base line is seen both above and below the boundary, if the boundary is clear of the meniscus and the bottom of the cell, and represents uniform concentration. For rough work, the base line may be sketched in through the boundary region, but for accurate work an experimentally determined base line must be used. The base line suffers distortion of two kinds:

(a) Curvature of the cell end plates produces a curvature of the base line (Fig. 5) and sedimentation of buffer solutes may also produce a distortion. It is therefore necessary to

Fig. 5. Photographic records of boundary and base line.

obtain a base line, corresponding with each record of the boundary whose area it is wished to measure, using buffer of identical composition and under the same conditions of rotor speed, temperature, time (we have found this unimportant with acetate buffer), degree of filling of the cell and optical settings.

(b) The base line, obtained as described in (a), fits the boundary diagram only *above* the boundary (Fig. 6). This

is due to the different refractive indices of the solution below the boundary in the experimental run (where it contains protein) and the base line run (where it contains only buffer). This difference, since the cell end plates are distorted to form a lens, alters the focal length of the optical system, which causes the base line to change in slope.

We have, therefore, used only diagrams in which a considerable length of base line is visible both above and below the boundary, and have fitted the tracing of the experimentally determined base line to the regions above and below the boundary, giving them the requisite slopes, and allowing intersection of the two parts of base line to occur in the middle of the boundary (Fig. 6). This procedure appears to give a fair approximation of the true area.

Fig. 6. Tracings from photographic records: (a) boundary tracing, (b) base line fitted above the boundary, (c) base line fitted below the boundary.

The area between the 'peak' and the fitted base line was measured with a planimeter, making five complete circuits of the area ($P \text{ cm.}^2$). The planimeter was standardized with the radius arm supplied with it; its wheel was always run on a standard paper surface. The magnification of the enlarger (M_E) was obtained by measuring the meniscus-index distance on the plate by a travelling microscope and on the tracing by a ruler (to 0.5 in 120 mm.). The magnification of the spherical schlieren lens (M_s) was measured as already described, and that of the cylindrical lens (M_c) by measuring the photograph through it of an accurately made rectangular aperture; the distance (a) from the focusing lens on the rotor casing to the diagonal edge (equal to its focal length) was measured by ruler (to 0.5 in 133 cm.); the cell

Table 3. *Measurement of boundary diagrams of lactoglobulin*

(Lactoglobulin 1%: total refractive increment 182×10^{-5} . Cell thickness 1.219 cm.)

Time from reaching full speed (min.)	Angle of schlieren edge ($^\circ$ from vertical)	Estimated refractive increment $\times 10^5$ (N)	N as % of total
68	25	184	101
78	25	173	96
88	25	184	102
98	30	181	100
108	30	182	101
110	35	173	96
118	35	176	98
		Mean $179 \pm (\text{s.d.}) 4.9$	98.4 ± 2.7

(Lactoglobulin 0.526%: total refractive increment 94.7×10^{-5} .)

82	40	92	97
102	40	96	101
112	45	92	97
122	45	95	100
132	50	97	102
		Mean $94.4 \pm (\text{s.d.}) 2.3$	100 ± 2

thickness (b) was measured by micrometer, the thickness of the quartz plates having been measured before assembly. The refractive increment N is then given by

$$N = \frac{P \cot \psi}{5M_s^2 M_s M_o ab} \left(\frac{A - I + x}{A - I} \right)^2,$$

where ψ is the angle of the diagonal edge from the vertical. The square term allows for the dilution of protein through sedimentation in a non-uniform field and in a sector-shaped cell (Svedberg & Pedersen, 1940).

According to this procedure, measurements have been made of the boundary diagrams obtained in two of the experiments on lactoglobulin described above. The results are given in Table 3.

DISCUSSION

The boundary diagrams obtained with lactoglobulin are ideal for accurate integration; an accuracy of about 2.5% (S.D.) was obtained from measurement of a single diagram, and greater accuracy is obtainable by taking the mean value from several diagrams of a given run (measurement of six give an accuracy of about 1%). The error will be greater than this in the following cases: (1) where the boundary is very broad, leaving too little base line for accurate fitting; (2) where it is very narrow, so that the area to be measured is small and the distance moved during an exposure is an appreciable fraction of its width; (3) where one boundary is incompletely resolved from another, or where heterogeneous material distorts the apparent base line; (4) where the use of a high optical sensitivity (with dilute solutions) lessens the sharpness of the line of demarcation in the diagram.

In the course of this work it was noticed that, for a period, wrong values had been obtained for the quantity of sedimenting components, through failure

to take into account the fact that the focusing lens then in use on the rotor casing was not placed at its focal distance from the diagonal edge. A correction of 7% is to be added to the values given in the following publications: Ogston (1945), Ogston (1946) and Johnston & Ogston (1946); application of this correction to data given in the last for serum albumin and lactoglobulin gives a mean integration value of 97%.

The accuracy of the Philpot optical system is thus shown to be little inferior to that of the scale method of Lamm (1934).

SUMMARY

1. A systematic investigation of the accuracy of measurement of sedimentation constants by means of the Svedberg oil-turbine ultracentrifuge has shown that values reproducible to 0.5%, and probably accurate to the same limits, can be obtained if the conditions of running are standardized, if care is taken over measurement of the various quantities concerned, and if correction is made for error of measurement of the temperature of the cell contents. A new method of computing the sedimentation constant is given.

2. Examination of the accuracy of estimation of the quantity of a sedimenting component from the boundary diagram given by the diagonal-schlieren method of Philpot (1938) has shown that, under favourable conditions, a single measurement may have a standard deviation of 2.5%, making an accuracy of 1% attainable from the data of a single run. Special attention is needed to the fitting of the base line and an improved method of doing this is described.

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