7. 2-[8-^{14}C]Naphthylamine is absorbed from the bladder into the blood of a cat to a notable extent, but 2-amino-1-[8-^{14}C]naphthyl hydrogen sulphate is absorbed very much less readily.

We wish to thank Dr D. Manson, Institute of Cancer Research, Pollard's Wood, for advice on the extraction of the metabolic 2-amino-1-naphthyl hydrogen sulphate from dog urine. We are indebted to Mrs M. E. Ross, Miss P. A. Melhuish, Mrs R. Coombes and Mr R. G. Harrison for technical assistance.

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A Graphical Method for the Rapid Determination of Sedimentation Coefficients

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The calculation of sedimentation coefficients by the usual methods, though not particularly difficult, can be time-consuming, particularly if the systems examined in the analytical ultracentrifuge are complex. Various aids to this calculation, such as tabulated values of the various constants required, are in general use, but these save only a small amount of the mathematics required. The procedure described in this paper completely eliminates all calculation, and also serves as a convenient means for recording the data. In addition, the sedimentation coefficients obtained are based on an assessment of all the data available for each run. A system which can achieve this degree of simplification requires preparation, but it is hoped that this paper will enable this to be done with the least possible effort on the part of the user.

PRINCIPLE OF THE METHOD

The determination of a sedimentation coefficient (S) depends upon the solution of the equation

$$S = \frac{\ln (x_2/x_1)}{\omega^2(t_2 - t_1)}, \qquad (1)$$

where x is the distance of a boundary from the axis of a rotor rotating with an angular velocity of ω at time t (Svedberg & Pedersen, 1940).

Equation (1) shows that a graph of $\ln x$ against t, for a substance having a constant sedimentation coefficient, is a straight line having a slope which is a function of ω^2 and S. Consequently, if x is plotted on logarithmic graph paper, S may be read off this curve by means of a protractor calibrated in terms of rev./min. against S.

Both the graph paper and the protractors have been designed for use with the Spinco Model E ultracentrifuge (Beckman/Spinco Division, Palo Alto, Calif., U.S.A.), but there is no reason why similar apparatus should not be employed with other ultracentrifuges. This type of ultracentrifuge has a number of fixed speeds and fixed intervals between photographic exposures, and the number of possible combinations is very large, so that a certain amount of selection has had to be made. The speeds covered are from 21 740 to 59 780 rev./min. and times from 4 to 246 min., which covers adequately all S values from 1 to 250 s, and it is possible to extend this range considerably, as is explained below.

APPARATUS

The graph paper. Graph paper covering the range required is not available commercially at present, but it may be prepared fairly simply by one of several methods. It is conveniently included as part of a special data sheet, which

serves as a record of the run in question. The scale (Fig. 1) has as an abscissa time-intervals, eight in all, spaced 2 cm. apart. The ordinate is calibrated logarithmically in terms of x, from $x = 5{\cdot}9$ to $x = 7{\cdot}1$ by using a scale of 0·01 log units = 2 cm. (Table 1).

The ratio of the two scales is, of course, the important factor, but the dimensions given are convenient in use. This calibrated area (Fig. 1), which is approx. 16·1 cm. × 14 cm., may be printed on a sheet of duplicating paper 21 cm. × 33 cm. in size, which can contain the following information in addition: material under investigation, with concentration and composition of solvent; date; speed of centrifuge; exposure interval and time; revolution-counter readings; drive voltage; photographic material; solvent viscosity and density; corrected S; comments. Schlieren angles are recorded with exposure number, and the uncorrected S values are recorded beside the appropriate lines on the graph.

The graph paper may be made by drawing on a Durotype stencil No. 6 (Gestetner Ltd., London) with pen No. SP2 and a Gestetner writing sheet. Alternatively, it may be drawn in Indian ink on a sheet of paper and duplicated on a stencil by the Gestefax (Gestetner Ltd.) process, which is a dry electronic stencilling process and gives stable stencils. Copying by ordinary photographic processes is not sufficiently reliable because of the uneven stretching of the paper.

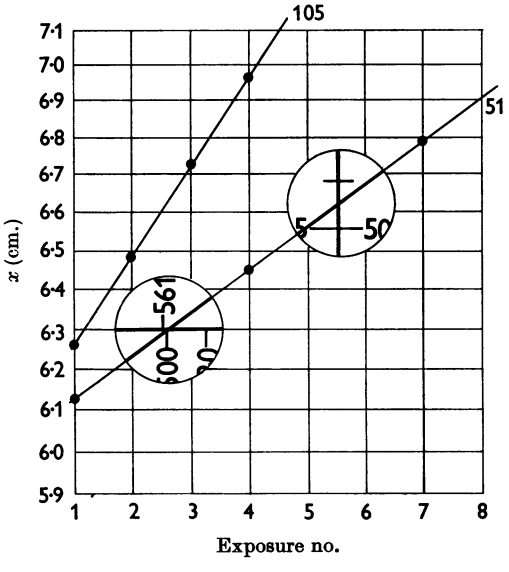

Fig. 1. The logarithmic graph paper, showing enlarged, in the circles, the use of the 4 and 16 min. protractor (Fig. 2). On the left, the lower protractor scale is fitted at 35 600 rev./min. to the intersection of one of the horizontal graph lines (6·3 cm.) and the experimental curve. On the right, the experimental curve is seen to intersect the protractor ordinate scale at $S = 51$ s. The data are taken from a run (4/1/60) made at 35 600 rev./min. and 21·1°, with 4 min. exposure intervals, of turnip yellow mosaic virus (upper curve: $S = 105$ s) and the nucleic acid-free protein (lower curve) in 0·1 M-KCl. Total protein concentration approx. 5 mg./ml.

Table 1. *Ordinate values for the logarithmic graph paper with abscissa intervals of* 2 *cm.*

x (cm.)	Ordinate (cm.)
7·1	16·08
7·0	14·85
6·9	13·60
6·8	12·33
6·7	11·04
6·6	9·72
6·5	8·41
6·4	7·06
6·3	5·68
6·2	4·31
6·1	2·88
6·0	1·46
5·9	0·00

Table 2. *Abscissa distances for* 4 *and* 16 *min. protractor, in units of* S *on the* 4 *min. ordinate*

A convenient size is given by 150 s = 100 mm. The 16 min. ordinate scale is one-tenth that of the 4 min. ordinate scale, and the abscissa figures have been adjusted to take this into account.

Rev./min.	4 min.	16 min.
21 740	184·7	—
23 150	163·0	—
24 630	144·0	—
25 980	129·5	—
27 690	113·9	—
29 500	100·4	—
31 410	88·5	—
33 450	78·1	—
35 600	69·0	172·4
37 020	63·8	159·5
39 460	56·1	140·3
42 040	49·5	123·6
44 770	—	109·0
47 660	—	96·2
50 740	—	84·8
52 640	—	78·8
56 100	—	69·4
59 780	—	61·1

Table 3. *Abscissa distances for* 8 *and* 32 *min. protractor, in units of* S *on the* 8 *min. ordinate*

The 32 min. ordinate is one-tenth the scale of the 8 min. ordinate. In order to make the two protractors of approximately equal size, a scale of 60 s = 6 in. is recommended. In this case, the figures in the table will be in 0·1 in. units.

Rev./min.	8 min.	32 min.
29 500	50·22	—
31 410	44·27	—
33 450	39·05	—
35 600	34·50	—
37 020	31·90	—
39 460	28·06	- -
42 040	24·73	—
44 770	21·80	—
47 660	19·24	48·1
50 740	16·96	42·4
52 640	15·77	39·4
56 100	13·88	34·7
59 780	12·23	30·6

The protractors. These are calculated from equation (1) by using values for the intervals between exposures of 4, 8, 16 and 32 min. For 4 min. exposures the abscissa distance to the left of the ordinate may be calculated for any centrifuge speed from the relationship

$$y = 8{\cdot}738 \times 10^{10}/(\text{rev./min.})^2 \text{ Svedberg units (s)}, \quad (2)$$

and the other scales are in proportion to the time intervals used.

A considerable simplification of the construction of the protractors may be effected by using two ordinate scales in the proportion of 10:1, so that only one set of ordinate calibrations is necessary. This has been done in both pro-

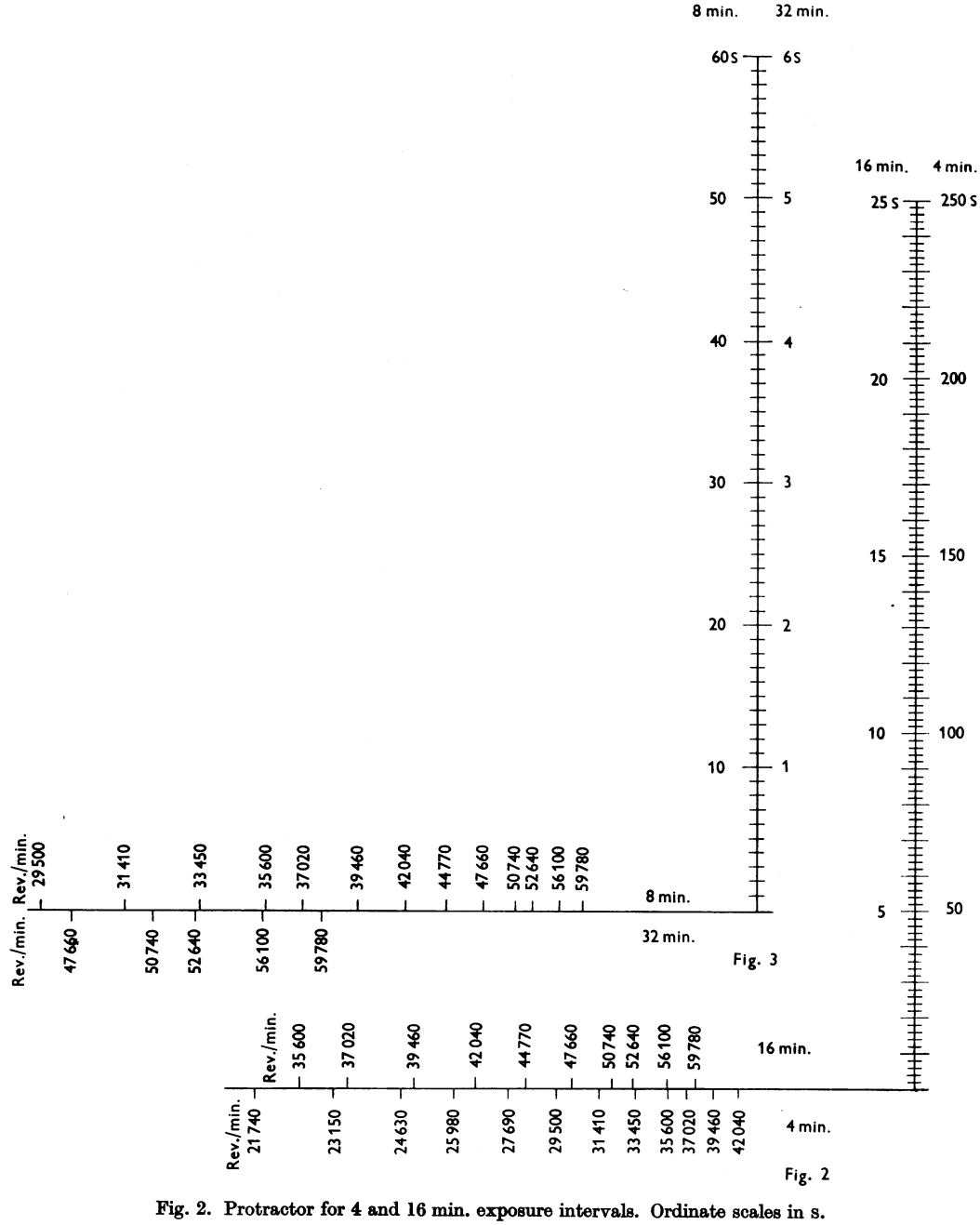

Fig. 2. Protractor for 4 and 16 min. exposure intervals. Ordinate scales in s.

Fig. 3. Protractor for 8 and 32 min. exposure intervals. Ordinate scales in s.

tractors in combination with a fourfold difference in the time intervals used. The abscissa scales are then in the proportion of 2·5:1. These protractors are illustrated in Figs. 2 and 3. The data used in making them are given in Tables 2 and 3, which also include an indication of a convenient size for each protractor, though this may be varied to suit any particular requirement.

The actual protractor scales illustrated cover the range of s values most commonly encountered, and they may be extended by a number of devices. For example, the 4 min. scale, if used with 2 min. exposure intervals in the ultracentrifuge, will give half the correct S value, and so S values as high as 500s may be determined. Furthermore, the low gear-speeds from 3397 to 9341 rev./min. (numbered in red) may be used in conjunction with the corresponding normal speeds (numbered in white), when the S value determined from the protractor needs to be multiplied by 36 (the square of the gear-box ratio). This extends the range of values which may be measured to 9000s.

It is important in preparing the scales that a stable material should be used. Ideally they should be drawn on card at least twice the size required, and reduced photographically onto a process-type cut film (Kodaline KS3, Kodak Ltd., London). Both protractors (Figs. 2, 3) can be incorporated onto the same sheet of film. The most important consideration in preparing the protractors is that the ordinate and abscissa scales should be exactly at right angles to each other.

HOW TO USE THE METHOD

The experimental values of x are read off from the ultracentrifuge plates in the usual way, and plotted on the logarithmic graph paper (Fig. 1). The appropriate protractor (Fig. 2 or 3) is then placed on the graph with its abscissa scale along a horizontal graph line (in Fig. 1, left, this is on line $x = 6{\cdot}3$ cm.), with the line corresponding to the rotor speed at the intersection of the experimental curve with the latter. The experimental curve will then intersect the ordinate scale of the protractor at the required value of S (Fig. 1, right).

The objective of the system described above was first and foremost convenience. It is quite obvious that the problem may be solved by using simpler equipment, e.g. by making the protractor scales integral with the graph paper, and using parallel scales to transfer the slopes, but it was thought that this method was not quite so convenient in use.

As far as accuracy is concerned, it has been found in practice that results are reliable within about ± 1s in 100s if boundary positions are estimated to within 0·01 cm. The step involving the plotting of log x against t is particularly valuable because it serves as a check that sedimentation is progressing uniformly, besides ensuring that the value for S is based on a weighted assessment of all the experimental observations. If two or more components are present during a run, it is possible to check that the two curves intersect at the level of the meniscus; and, if a peak is just resolved at the end of a run, it is possible to draw a line from its position to the meniscus, and so estimate the slope accurately.

SUMMARY

1. A graphical method for the evaluation of sedimentation coefficients is described. Its principle involves the employment of a special logarithmic graph paper and protractors calibrated directly in Svedberg units.

I should like to thank Mr S. Frey for the trouble which he has taken with the photographic problems involved.

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The Reactions of some Aromatic *C*-Nitroso Compounds with Haemoglobin

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Although the reactions of many ligands with methaemoglobin have been described, work with haemoglobin has, until recently, been confined to the low molecular weight molecules of oxygen, carbon monoxide and nitric oxide. St George & Pauling (1951) have, however, examined the equilibrium between haemoglobin and a series of *iso*-cyanides, finding a sharp decrease in affinity on passing from *iso*propyl *iso*cyanide to *tert.*-butyl *iso*-cyanide. With free haem, on the other hand, they found much smaller differences in affinity. To explain their results, they put forward the idea that the haem groups of haemoglobin are located in a 'crevice' in the protein which hinders the approach