LETTERS TO THE EDITORS

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A New Method for Measuring Diffusion Constants of Biologically Active Substances

THE study of diffusion constants of proteins and other materials of high molecular weight has added much to our knowledge of the nature of these substances (Svedberg and Pederssen[1], Polson[2]). These studies were made on materials which were obtained in the pure state. Unfortunately, when dealing with substances comprising several components, the usual optical method of diffusion (Lamm[3]) cannot be applied except with great difficulty. In such cases recourse must be made to analytical methods. The method of Northrop and Anson[4], namely, diffusion through a porous plate, has been of great value, but this method too has its limitations. Bourdillon[5] has proposed a method for the analytical determination of diffusion constants. His method, although theoretically sound, is very difficult to apply, especially when dealing with viruses. (For a criticism of the above-mentioned methods, see Markham, Smith and Lea[6].)

An account is given below of a method which has been found suitable for the measurement of the diffusion constant of horse-sickness virus.

Theoretical: From the well-known law of Fick

$$ds = -AD\frac{dc}{dx}\cdot dt,$$

and its solution

$$\frac{dc}{dx} = \frac{C_0}{2\sqrt{\pi Dt}}e^{-x^2/4Dt},$$

the following equation has been deduced

$$D = \frac{S^2}{C_0^2A^2}\cdot\frac{\pi}{t},$$

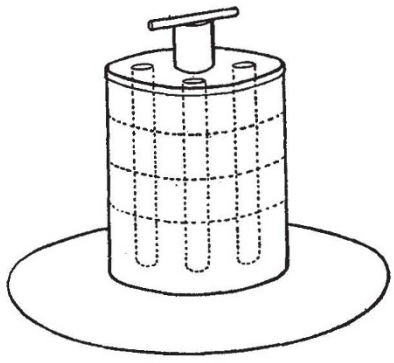
where S is the amount of substance which diffused through a cross-section A cm.^{2} at the original boundary $X = 0$ in t sec. D is the diffusion constant and C_0 is the original concentration.

This equation can be applied to all substances which do not sediment appreciably under the influence of gravitation, such as the proteins, enzymes and the small viruses. Its range does not include the larger viruses like vaccinia and psittacosis, as their sedimentation constants are too high.

Experimental: To measure the amount of substance which passed a given layer in a diffusion vessel, the apparatus shown in the accompanying diagram was made. Four circular metal sections 1 cm. thick and 4 cm. in diameter were cut and holes 0·5 cm. in diameter drilled through them, except the bottom section, into which the holes extend three-quarters of the thickness of the section. The flat faces of these sections were well ground. They turn on a central bolt which is fixed in a wooden base. In a certain position the holes coincide to form four cylindrical cavities 4 cm. long and 0·5 cm. in diameter. The surfaces between the sections were smeared with a thin layer of wool grease.

The diffusion experiment was run as follows.

The virus solution was placed in the four cavities formed by the bottom two sections, and the cavities in the top two sections filled with suspension medium of a slightly lower density than the fluid in the bottom



cavities. The apparatus was placed in a constant-temperature room and when temperature equilibrium had been reached the top two sections were turned slowly so that the cavities corresponded with those in the lower two sections. In this way sharp interfaces were formed at the contact of the top fluid with the bottom fluid. The apparatus was left in that position for definite periods of time, after which the top fluid was again isolated by rotating the segments. The fluid in the top sections was sucked out with a syringe, the volumes measured and tested for virus content.

The accompanying table gives the results obtained:

Exp. No.	Time (sec.)	A (cm.^{2})	C_0 in mid.* per c.c.	S in mid.*	$D \times 10^7$ cm.^{2}/sec.	
1	151,200	0·2	160,000	2232	1·01	Av. 0·80
2	151,200	0·2	440,000	6480	1·01	
3	237,600	0·2	226,000	3360	0·73	
4	331,200	0·2	200,000	2796	0·46	

* Determined by the Read and Münch method of 50 per cent end-points.

The diameter calculated from the Stokes-Einstein formula for the diffusion of a substance gave the value $d = 53·2\ \mu\mu$. This value agrees very well with those determined by other means, namely, ultracentrifugation $45·4\ \mu\mu$, and ultrafiltration 40–60 $\mu\mu$ (Polson[7]).

From the sedimentation constant $S = 286 \times 10^{-13}$ Svedbergs, the specific volume $V = 0·8$ and the diffusion constant $D = 0·8 \times 10^{-7}$ cm.^{2}/sec., the molecular weight is calculated according to the formula of Svedberg[1],

$$M = \frac{RTS}{D(1-V\rho)} = 44{,}500{,}000,$$

where ρ is the density of the suspension medium.

This value for the molecular weight must be considered approximate, as the determination of D depends on the relationship $\frac{(S)^2}{(C_0)^2}$, the determination of which could only be done by biological means.

A more extensive article will be published in the *Onderstepoort Journal*.

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[1] Svedberg and Pederssen, "The Ultracentrifuge" (Oxford University Press, 1940).
[2] Polson, *Koll. Z.*, **87**, 149, and **88**, 51 (1939).
[3] Lamm, *Nova Acta Reg. Soc. Scient. Upsal.*, **4**, 10, No. 6 (1937).
[4] Northrop and Anson, *J. Gen. Physiol.*, **12**, 543 (1928–29).
[5] Bourdillon, *J. Gen. Physiol.*, **24**, 459, and **25**, 263 (1941).
[6] Markham, Smith and Lea, *Parasitology*, **34**, 315 (1942).
[7] Polson, *Onderstepoort J. Vet. Sci. and Animal Ind.*, **16**, 33 (1941).