a different equilibrium and, probably, does not indicate a basic difference in the mechanism of lipid transport. For these reasons, it does not appear unreasonable to apply some of the findings obtained in the diabetic dog to diabetic man and suggest, tentatively, that a strict control of diabetes must be adopted to prevent those lipemic abnormalities suspected of having a pathogenetic relationship to atherosclerosis.

Summary. 1) Serum lipoproteins of normal and depancreatized dogs have been studied by means of zone electrophoresis on filter paper. 2) Dog serum contains 3 main groups of lipoprotein fractions: a fast-migrating albumin-α-globulin group, a slow-migrating β-globulin group and a β_2-γ-globulin group which does not migrate at all. 3) The bulk of serum lipids is carried by the albumin-α-globulin fractions and only a small amount by the β-globulins. This is the opposite of what happens in normal man. 4) Serum of depancreatized dogs under insulin control contains more total cholesterol and total lipid P than normal serum. The $\frac{\text{cholesterol}}{\text{lipid P}}$ ratio is increased. There is a tendency for an increase in amount of the slower migrating components of albumin-α-globulin fraction at the expense of the faster ones. 5) Withdrawal of insulin is followed by an immediate uniform increase of all lipoprotein fractions. As decompensation sets in, the β-globulin fraction increases out of proportion with other fractions. Thus, in decompensated diabetic dogs, as in man, the β-globulin fraction becomes the principal carrier of serum lipids. 6) The possible significance of these findings is discussed.

1. Fasoli, A., *Lancet,* 1952, v1, 106.
2. ———, *Acta Med. Scand.,* 1953, v145, 233.
3. Kunkel, H. G., and Slater, R. J., *J. Clin. Invest.,* 1952, v31, 677.
4. Fasoli, A., Ed. Premio Ganassini, Milano, 1953.
5. Fasoli, A., and Bonelli, M., *Minerva Med.,* 1951, v42 (2nd part), 402.
6. Snavely, J. R., Goldwater, W. H., Randolph, M. L., and Unglaub, W. G., *J. Clin. Invest.,* 1952, v31, 664.
7. Gofman, J. W., Jones, H. B., Lindgren, F. T., Lyon, T. P., Elliott, H. A., and Strisower, B., *Circul.,* 1950, v2, 161.
8. Keiding, N. R., Mann, G. V., Root, H. F., Lowry, E. Y., and Marble, A., *Diabetes,* 1952, v1, 434.
9. Hanig, M., and Lauffer, M. A., *ibid.,* 1952, v1, 447.
10. Foà, P. P., Weinstein, H. R., and Smith, J. A., *Am. J. Physiol.,* 1949, v157, 197.
11. Durrum, E. L., *J. Am. Chem. Soc.,* 1950, v72, 3943.
12. Flynn, F. V., and DeMayo, P., *Lancet,* 1951, v2, 235.
13. Bloor, W. R., Pelkan, K. F., and Allen, D. M., *J. Biol. Chem.,* 1922, v52, 191.
14. Horwitt, B. N., *ibid.,* 1952, v199, 537.
15. Fasoli, A., and Foà, P. P., unpublished data.
16. Page, J. H., Lewis, L. A., and Plahl, G., *Circul. Res.,* 1953, v1, 87.

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Particle Size of Yellow Fever Virus. (20969)

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Since the original ultrafiltration experiments on yellow fever virus by Bauer and Hughes (1) and the ultracentrifugation studies on the virus by Pickles and Bauer(2) no work on the measurement of this virus has been reported.

Recently the technic of particle size determination using the Spinco preparative ultracentrifuge became available Polson and Linder(3). This method which allows sedimentation constants to be determined with fair accuracy and without laborious preliminary purification was used to determine the particle size of the yellow fever virus.

Material. The 17D strain of yellow fever virus was used after it had received 3 intracerebral passages in adult mice in this laboratory. The brains of 4 mice moribund after intracerebral inoculation of neurotropic yellow fever were emulsified in 40 ml 10% rabbit serum saline, the material was clarified by centrifugation at 2700 r.p.m. in a refrigerated centrifuge, and 6 ml of supernatant mixed with an equal volume of 2% Caminella sincta haemocyanine in phosphate buffer at pH 7.0. The haemocyanine tends to reduce sedimentation irregularities caused by convection (Polson and Linder l.c.). It was experimentally confirmed that haemocyanine does not interfere with sedimentation nor does it affect the viability of the virus.

Methods. Samples of the mixture of yellow fever virus and haemocyanine were ultracentrifuged in the model L Spinco centrifuge for 100 minutes at rotor velocities ranging from 11000 to 25000 r.p.m., and the virus contents at different levels in the tubes determined by titration in mice. Prior to sampling in each case the position of the haemocyanine boundary in the centrifuge tube was carefully noted. The samples were titrated by intracerebral inoculation of 10-fold dilutions into groups of 6 3-4 weeks old mice, each mouse receiving 0.03 ml of fluid. The mice were kept under observation for 2 weeks. Titres are expressed in LD_{50}. The titres of the different samples were plotted against their mean relative position in the centrifuge tube, and the position of the sedimenting boundary determined from the graph (Polson and Linder l.c.). The level of the sedimenting boundary was taken to be that at which the virus concentration was 50% of that of the original emulsion. The sedimentation constant of the virus was calculated from an equation deducted from that of Svedberg and Pedersen (4):

$$S_2 = S_1 \frac{H_2}{H_1} \cdot \left(\frac{2X_1 + H_1 \sin \alpha}{2X_1 + H_2 \sin \alpha} \right)$$

where S_2 and S_1 are the sedimentation constants of the virus and the haemocyanine and H_2 and H_1 the respective distances which their boundaries migrated down in the tube during centrifugation. X_1 is the distance of the fluid meniscus from the centre of rotation of the rotor, and α is the angle of inclination of the centrifuge tube with the vertical. The value of S_1 is 100 Svedberg units.

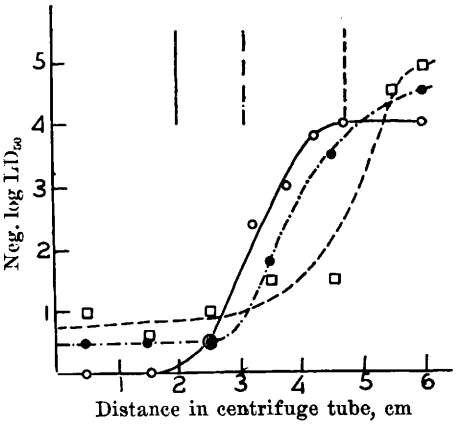

FIG. 1. Sedimentation diagrams of yellow fever virus, strain 17D, in 1% haemocyanine at pH 7.0. Vertical lines indicate position of haemocyanine boundary in different centrifugation runs. Values at 6 cm are titers before centrifugation. ——— = 18000 r.p.m. for 100 min. —·—·— = 19800 r.p.m. for 100 min. – – – – = 24400 r.p.m. for 100 min.

Results. It was found that after centrifugation at 11000 r.p.m. for 100 minutes very little sedimentation of the virus had occurred and at rotor velocities of 25000 r.p.m. for the same period the virus was centrifuged down completely. At 18000 r.p.m. to 20000 r.p.m. the virus boundary was in suitable positions in the tube to allow calculation of the sedimentation constant. In Fig. 1 are given the sedimentation diagrams obtained at different rotor velocities together with the positions occupied by the haemocyanine in the different runs. It will be noticed that the virus boundaries were well ahead of the haemocyanine boundaries and that the upper layers of fluid in the centrifuge tube were almost completely freed of virus by the centrifugation. Table I records the results of the 3 experiments. The sedimentation constant calculated from equation (1) is 184 S for the run at 18000 r.p.m., and 157 S for that at 19800 r.p.m. From these values the particle size was calculated using the modified Stokes equation:

$$r^2 = \frac{9}{2} \frac{S\eta}{(d-\rho)}$$

TABLE I. Experimental Data of 3 Centrifugation Runs of Mixtures of Haemocyanine and Yellow Fever Virus Strain 17D. $a = 26°$. $X_1 = 5.32$ cm. $S_1 = 100$ Svedberg units.

r.p.m. (×100)	H_1, cm	H_2, cm	LD_{50} of samples taken at cm: 0.5	1.5	2.5	3.25	3.5	3.75	4.25	4.5	4.75	BL	Original, 6 cm
180	2.0	4.0	0	0	0–1	2.4	—	3.0	3.8	—	4.0	—	4.0
198	3.1	5.25	0–1	0–1	0–1	—	1.8	—	—	3.5	—	—	4.5
244	4.75	B	1	.6	1.0	—	1.5	—	—	1.5	—	4.5	4.9

B = bottom; BL = bottom layer.

where r is the radius of the particle, S the sedimentation constant, η the viscosity of water at 20°C, d the density of the virus particle assumed to be 1.33 g/cc and ρ the density of water at 20°C. The diameter of the particle determined in this way ranges from 29.2 to 31.4 mμ.

Discussion. The size of yellow fever virus particle as determined in this work is nearer that found by Bauer and Hughes (l.c.) in ultrafiltration experiments than that recorded by Pickles and Bauer (l.c.).

Summary. The particle size of the 17 D strain of yellow fever virus has been determined as ranging from 29.2 to 31.4 mμ by the method of ultracentrifugation coupled with biological assay.

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1. Bauer, J. H., and Hughes, T. P., *Am. J. Hyg.*, 1935, v21, 101.

2. Pickels, E. G., and Bauer, J. H., *J. Exp. Med.*, 1940, v71, 703.

3. Polson, A., and Linder, A. M., *Bioch. et Bioph. Acta*, 1953, v11, 199.

4. Svedberg, T., and Pedersen, K. O., *The Ultracentrifuge*, Oxford, Clarendon Press, 1940.

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Influence of Alpha-Tocopherol upon Development of Cardiovascular Necrosis and Hypertension in the Rat.* (20970)

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Medical literature is replete with contradictory reports on therapeutic efficacy of vit. E. Thus large dosages of alpha-tocopherol or mixed tocopherols have been reported to exert therapeutic effects in muscular dystrophies and rheumatic diseases of men(1-3). Also that prolonged administration of this vitamin may bring about dramatic amelioration in hypertensive cardiovascular disease, as well as many peripheral vascular conditions(4-6). However, well controlled clinical studies by others failed to confirm the reported benefits(7-10). The vast animal experimental literature dealing with vit. E contains surprisingly few references to the beneficial action of this vitamin, if one takes exception to specific effects in induced deficiency. With regard to the cardiovascular system, only 2 publications described therapeutically desirable effects. Allardyce and associates(11) reported a hypotensive effect of vit. E in desoxycorticosterone induced hypertension of albino rats, and Holman(12) was able with the help of vit. E to inhibit emergence of experimental necrotizing ar-

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