of the first generation animals after 1 year was: dextrose-fed 1.9% (6 animals), sorbitol-fed 3.5% (7 animals), and arlex-fed 3.8% (3 animals). Animals from this group previously fasted for 48 hours showed the following values for liver glycogen: dextrose-fed 0.33% (10 animals), sorbitol-fed 0.44% (6 animals), and arlex-fed 0.22% (4 animals). Grossly and histologically the kidney, liver, spleen, pancreas, and duodenum of these animals in the 3 generations showed no abnormalities deemed attributable to the dietary regimen.

Summary. Comprising 5% of the food intake, neither sorbitol nor arlex affects deleteriously the rate of growth, liver-glycogen storing capacity or important metabolic viscera of the white rat through 3 generations.

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Size of Infective Particle and Hemagglutinin of Influenza Virus as Determined by Centrifugal Analysis.

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It has recently been shown[1] that sedimentation boundaries could be obtained and approximate size determinations made with dilute protein suspensions in the angle centrifuge if a synthetic density gradient of sucrose or some other nonsedimentable material was added to counteract disturbances of convection. Under these conditions tubes could be removed from a centrifuge and sampled without greatly altering a boundary. These principles have been applied to the sedimentation of influenza virus in allantoic fluid, and the findings are herewith reported.

The PR8 strain of influenza A virus or the Lee strain of influenza B virus was inoculated into the allantoic sacs of 11-day-old White Leghorn embryos. After 48 hours' incubation at 37°C the eggs were chilled overnight at 4°C; the blood-free allantoic fluids were removed and cleared by centrifugation at 1800 r.p.m. for 5 minutes. Without further treatment the virus fluids were spun in celluloid tubes in a vacuum air-driven centrifuge in the presence of a sucrose density gradient with the concentration of sugar increasing from a negligible amount at the top of the fluid column to nearly 12% at the bottom of the tube. After centrifugation at a given rate 1 cc samples were removed at different levels in the fluid column by means of a sampling apparatus already described.[2] Control tubes which had been prepared in identical manner but not centrifuged were allowed to stand for a comparable time and also sampled. All the samples were immediately tested for infectivity in mice and for capacity to agglutinate chicken red blood cells.[3]

The results of a typical experiment are shown in Fig. 1. Repeated tests with allantoic fluids containing either A or B virus strains after centrifugation at 12,800 r.p.m. for 28 minutes showed the following characteristics: (1) a marked drop in virus titer in the upper cc of the fluid column to about 0.5% of the original concentration, (2) a sharp rise in virus concentration (a boundary indicating sedimentation of a large group of virus particles of about the same size) slightly more than one-third of the way down the tube followed by (3) a region of uniform or very gradually increasing concentration, and (4) a marked increase in concentration in the bottom sample due to deposition of the virus. The displacements of the boundary from the meniscus were roughly proportional to the amount of centrifugation. For example, the sedimenting virus boundary moved only one-sixth of the tube length when the centrifuga-

1 Pickels, E. G., *J. Gen. Physiol.*, 1943, **26**, 341.

2 Hughes, T. P., Pickels, E. G., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1938, **67**, 941.

3 Hirst, G. K., *J. Exp. Med.*, 1942, **75**, 49.

tion time was decreased to 14 minutes, whereas when increased to 56 minutes at the same speed it moved almost to the bottom of the tube. In the latter instance careful sampling

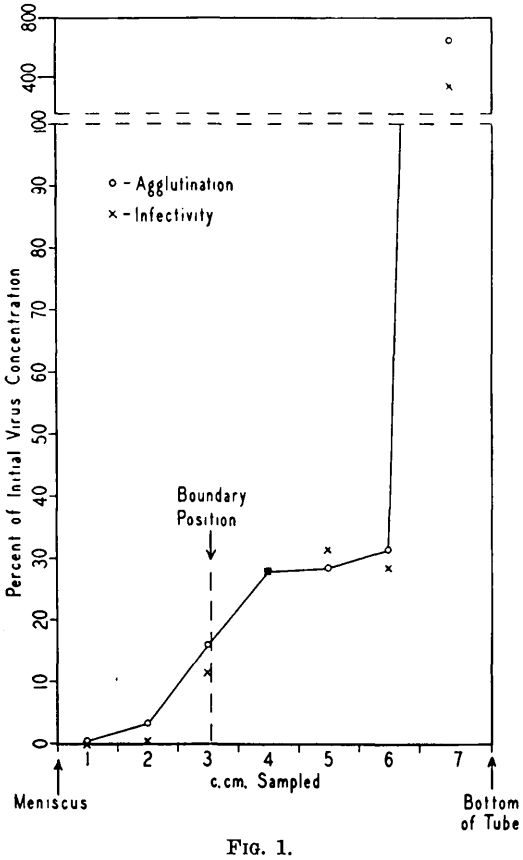

FIG. 1.
Sedimentation of infective particle and hemagglutinin of influenza B virus in allantoic fluid. Samples were taken from celluloid tubes (diameter = 1.3 cm) inclined at 35° to axis of rotation, after 28 minutes at 12,800 r.p.m. Meniscus of fluid was 6 cm from axis of rotation.

revealed that less than 0.1% of the original concentration of virus remained in the upper cc of the fluid column as measured by infectivity tests.

Although the sedimentation rate of boundaries obtained with allantoic fluids containing either A or B virus strains was approximately the same, differences in the character of the sedimentation curves were observed which suggest varying amounts of aggregation. The sucrose solutions had no detectable effect on the virus controls. Comparable tests in which allantoic fluids were centrifuged without a density gradient showed more virus (several per cent of the original concentration) in the upper part of the tube and a gradual increase in virus concentration down the tube without exhibiting a sedimenting boundary.

Determinations of the approximate particle size of influenza virus were made from measured displacements of the boundary from the meniscus after several different centrifugation times and by application of an equation already derived for the angle centrifuge.[1] Assuming the usual protein density value of 1.33, the length (or diameter, if spherical) of the influenza virus particle in allantoic fluid was found to be at least 60 mμ. Even if an abnormally high density value is assumed, the size determination is altered by only a few percent. Confirmation of the sedimentation rate measured in the angle centrifuge was obtained by absorption (Fig. 2) and refractive

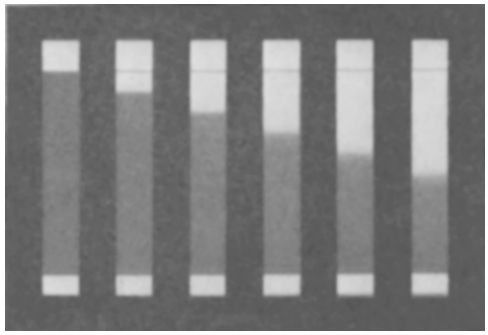

FIG. 2.
Absorption photographs showing sedimenting boundary of influenza B virus obtained from allantoic fluid. Speed 6300 r.p.m.; interval between photographs 10 minutes. Virus purified and concentrated by adsorption and elution from chicken red blood cells and subsequent centrifugation.

index photographs taken with concentrated and purified virus preparations in the ultracentrifuge. These results indicate that the actual size of influenza virus in allantoic fluid is nearer the value reported in the original filtration experiments with mouse lung extracts (80-120 mμ)[4] than that recently ascribed to the virus in allantoic fluid (10-15 mμ).[5] The physical properties of the virus will be more fully described in later reports.

4 Elford, W. J., Andrewes, C. H., and Tang, F. F., *Brit. J. Exp. Path.*, 1936, **17**, 51.

5 Chambers, L. A., and Henle, W., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 481.