benzoic acid to the action of sulfasuxidine and sulfaguanidine in the alimentary tract needs to be investigated on a quantitative basis. As shown by Wood,[12] working with 6 different sulfonamides and pure cultures of *B. coli*, the bacteriostatic potency of a sulfonamide is directly proportional to its ability to counteract the antibacteriostatic action of *p*-aminobenzoic acid. If, therefore, sulfasuxidine should happen to be appreciably more bacteriostatic than sulfaguanidine a greater amount of *p*-aminobenzoic acid might be required to exert a comparable antibacteriostatic action when the former is fed. Since Martin[10] has found that biotin improves the growth of sulfaguanidine-fed rats receiving *p*-aminobenzoic acid it must be concluded that, at the levels fed, the antagonistic action of the latter is not complete in the case of sulfaguanidine. Similarly our studies indicate that sulfasuxidine lacks considerable of being totally inhibited by *p*-aminobenzoic acid even at the seemingly large levels we have fed.

Summary. Biotin deficiency occurs in rats fed a purified diet containing sulfasuxidine and *p*-aminobenzoic acid. Growth appears to be a sensitive indicator of biotin deficiency in the rat.

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Sugar Alcohols. XXIII. Effect of Sorbitol Feeding on Successive Generations of Rats.

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In former studies[1] two of the authors showed mannitol and sorbitol to be innocuous when fed in comparatively large amounts to the *Rhesus macacus* monkey and man over long-time periods. The laxative action of large quantities of mannitol limits its value as a dietary adjunct. Sorbitol, however, does not possess this disadvantage and at present is enjoying a widespread use as a carbohydrate-like item of diet. It seemed fitting, therefore, to study the effect of sorbitol feeding on successive generations of animals.

Material and Methods. Crystalline sorbitol was used in one group of studies. In the other, a commercial sorbitol syrup was employed. This syrup is a non-crystallizing aqueous solution of sorbitol having a polyhydroxylic compound content of about 83%. It is manufactured by the electrolytic reduction of glucose and is known under the trade name of "Arlex."* Each of these substances mixed with Purina chow meal was fed to young white rats of our laboratory stock and also animals of the Duke University strain. Controls were fed dextrose and the basal diet. Each compound composed 5% of the total food intake. Males and females were kept separated until they were at least 100 days old and then mated. This was repeated through 3 generations. Growth curves of the rats fed the 3 types of food in the 3 generations were plotted. Periodically animals were killed for gross and histologic study. At the end of 1 year the capacity of the livers to store glycogen was determined. Eighty, first generation animals fed the experimental diet from the time of weaning were used and the experiment was extended over 20 months.

Results. The growth curves of the first generation animals fed the 3 kinds of food were identical within the normal variations of the experiment. The offspring of these animals showed similar growth rates in the second and third generations. The litter sizes were about the same. The average liver-glycogen[2] content

1 Ellis, F. W., and Krantz, J. C., Jr., *J. Biol. Chem.*, 1941, **141**, 147.

* Generous supplies of crystalline sorbitol and "Arlex" were furnished by the Atlas Powder Co. of Wilmington, Del.

2 Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 485.

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.