aqueous solutions of drug or of drug and of BAL. The lethal effects of the other 2 antimonials used by these authors, Fuadin and Neostam, contrary to their results, were found clearly to be enhanced by the administration of BAL. Similarly, BAL increased the lethal action of poisonous doses of Neostibosan. The dimercaptan did not alter the death rate from large intraperitoneal doses of Stibanose. The doses of BAL, whether dissolved in peanut oil or in water, were sufficient to save the lives of animals receiving lethal doses of either mercuric chloride or sodium arsenite. BAL in oil was administered in doses well below levels which by themselves are followed by any manifestation of poisoning. The largest intraperitoneal dose of aqueous solution of BAL used (about 60 mg per kg) can cause central stimulation in some rats, as shown by generalized muscular hyperactivity; however, this dose, followed by 30 mg per kg 4 hours later, can be given daily for at least 3 days without killing any animals.

Other experiments will have to be performed to determine, if possible, what is the explanation of the increased toxicity of Fuadin, Neostam and Neostibosan, if BAL be administered a short time later. The increased mortality associated with BAL-treatment occurred mainly within 24 hours; a significant difference in mortality was evident after 6 hours. It is possible that an acutely toxic complex substance is formed *in vivo* by the interaction of the dimercaptan and any of the 3 antimonials which themselves are not well-defined compounds; however, it is more acceptable to interpret the increased mortality as a summation of toxic effects. It seems reasonable to conclude that the favorable action of BAL in rats acutely poisoned by tartar emetic is the result of the formation of one or more mercaptides of antimony much less toxic than tartar emetic, just as is the case when an arsenical or a mercuric salt is rendered innocuous by the administration of BAL.

Summary. In rats, the acute lethal action of tartar emetic is significantly reduced by the administration of BAL. BAL increases the mortality rate in rats receiving Fuadin, Neostam or Neostibosan. The mortality rate in rats given large doses of Stibanose is not reduced by BAL.

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Electron Micrographs of Bacterial Cultures Infected with Bacteriophage.

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Now that we have learned to recognize individual virus particles through the electron microscopy of purified suspensions it has become practical to approach the more important question of how these particles are produced within the cells they infect. Probably bacteria with their bacteriophage provide the simplest virus system immediately accessible to existing technics. This is partly because sperm-like bacteriophage particles[1,2] can easily be recognized even in the presence of other particles of similar submicroscopic size and partly because bacteria are hosts small enough to allow an electron microscopic examination of both their surface and their internal structures.

To gain a satisfactory understanding of the steps involved in bacteriophage-production it is essential to be able to photograph sensitive bacteria under the conditions in which they grow and become infected. We have found that this can be done by studying replicas made of the surfaces of agar

[1] Luria, S. E., Delbrück, M., and Anderson, T. F., *J. Bact.*, 1943, **46**, 57.

[2] Sharp, D. G., Taylor, A. R., Hook, A. E., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 259.

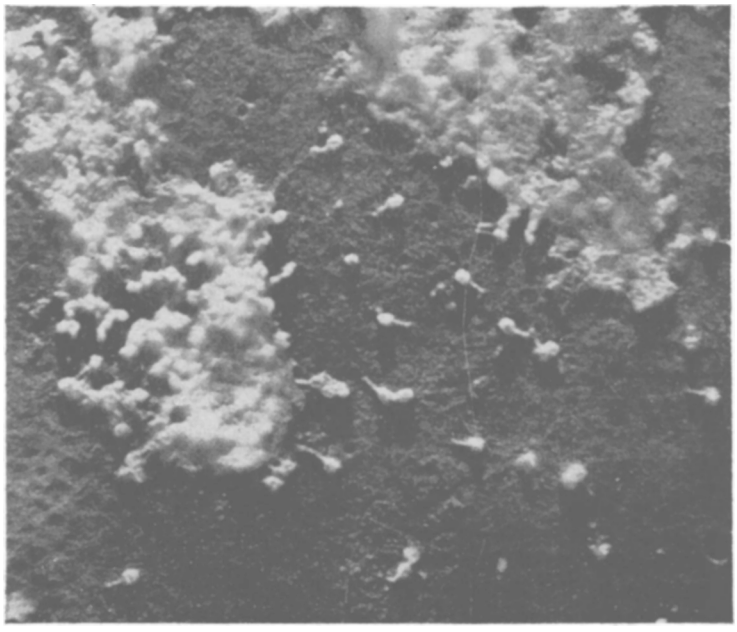

FIG. 1.

An electron micrograph of a gold-shadowed collodion replica of the surface of an *E. coli*-bacteriophage culture. The clear area of the plaque, besides showing a few adhering bacteriophage particles, is covered with pits which presumably are replicas of the heads of other particles. The finer granulation of this and the succeeding photographs is the macromolecular texture of collodion as brought out by the shadowing. Magnification *ca.* 19000 ×.

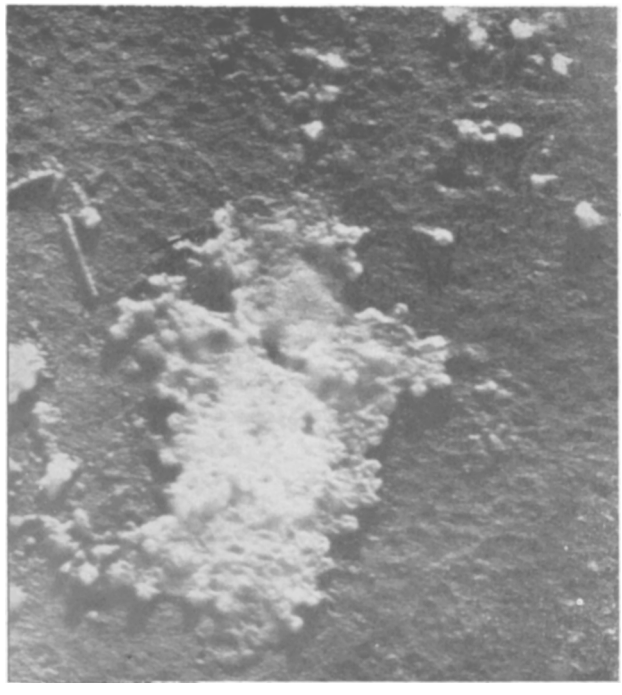

FIG. 2.

A clump of bacteriophage particles just inside the edge of a plaque area on a Petri dish culture. Magnification *ca.* 24,000 ×.

FIG. 3.
Clumps of bacteriophage filling the approximate outlines of two recently divided bacteria. Magnification *ca.* 35,000 ×.

plates incubated for various periods of time after inoculation with bacteria and bacteriophage. The technic we have been using to obtain such replicas is essentially the same as that recently described by Hillier and Baker.[3] It has consisted in covering the surface of a bacteriophage-bacterial growth with a dilute solution of collodion or other plastic and floating the resulting film off onto a water surface for further manipulation. Like Hillier and Baker we have found that organisms are retained by the film made in this way but our preparations have also shown numerous impressions of individual bacteria. This has made it possible to see in a single film details of both surface and internal structure.

Formvar dissolved in ethylene dichloride will yield good replicas but best results have been obtained with collodion. Collodion, USP, diluted with 5 to 6 volumes of normal amyl acetate, carefully spread over the growth-surface and drained till dry has produced films of the proper thickness. The degree of moistness of the surface has had a dominant influence on both the quality and the usefulness of the plastic film. When it was too wet the replica has reproduced little besides micro-drops of water covering its surface; when it was too dry masses of bacteria too thick for observation adhered to the finished replica. Contrast for microscopy has been introduced into the replicas floated from these Petri dishes by shadowing[4] them obliquely with metal to a calculated thickness of ca 8A of gold or ca 40A of chromium. Such shadowed replicas have been so thin that they have often stretched and distorted under the electron beam especially in and about regions of bacterial replication. This could be minimized by lightly metallizing the finished shadowed replicas through the vertical deposition of a few angstroms' thickness of aluminum, chromium or beryllium.

[3] Hillier, J., and Baker, R. F., *J. Bact.*, 1946, **52**, 411.

[4] Williams, R. C., and Wyckoff, R. W. G., *J. Applied Physics*, 1946, **17**, 23.

Typical micrographs of replicas made in the way just described are shown in Fig. 1-3. They are from the periphery of plaques developed on Petri dishes inoculated with a mixture of *E. coli* and a T2 strain of its bacteriophage. A number of isolated bacteriophage particles adhering to the film are evident in the clear areas of Fig. 1; the film also shows numerous pits that are undoubtedly replicas of particles that remained on the agar. The mass at the left of this micrograph, which approximately outlines the remains of a single bacterial cell, is a typical example of how completely bacterial protoplasm becomes converted into bacteriophage particles after invasion by one of them. Similar masses within the confines of old cells are reproduced at higher magnifications in Fig. 2 and 3. The separate particles can readily be recognized even when, as is commonly the case, they have partially collapsed presumably as a consequence of drying. These pictures do not reveal in detail how the bacteriophage particles are formed but they do suggest that a careful study of films taken from cultures containing bacteria in various stages of infection may be expected to yield such information.

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Sugar Alcohols. XXV. Sorbitol and Sorbitan as Precursors of Liver-Glycogen in the Rat.*

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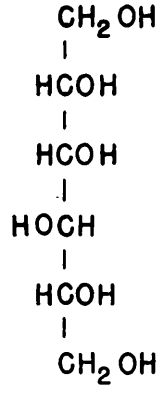
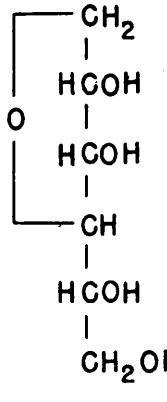
For many years we have been concerned with the fate of the sugar alcohols in the animal body.[1] Previously it was shown that mannitol served as a precursor of glycogen in the liver of the rat, whereas its anhydrides mannitan and isomannide were not available in this capacity. Sorbitol, an isomer of mannitol, surpasses the latter as a glycogen former. Having available crystalline sorbitan, anhydrosorbitol, it occurred to us to study its availability as a source of liver glycogen. Previously Carr and Krantz showed that polygalitol-1,5-anhydro-D-sorbitol was not available as a precursor of glycogen in the rat.[2] The relation of sorbitol to sorbitan is shown by the following formulas.

Experimental. Young white male rats from a uniform source, weighing from 150 to 200 g were fed a balanced ration (Purina Chow) for 3 days. They were then fasted for 48 hours and divided into the following experimental groups according to diet.

1. Cacao butter controls.
2. Cacao butter plus 30% crystalline sorbitol.



D-SORBITOL
M.p.97° $[\alpha]_D$ -1.97°



D-SORBITAN
Probable structure
M.p.113°

* The sorbitol and sorbitan were generously supplied by the Atlas Powder Company of Wilmington, Del.

1 Carr, C. J., and Krantz, J. C., Jr., *Advances in Carbohydrate Chemistry*, Vol. I, p. 175-192.

2 Carr, C. J., and Krantz, J. C., Jr., *J. Biol. Chem.*, 1934, **107**, 371.