GLYCINE—AN ESSENTIAL FACTOR FOR THE GROWTH OF BACTERIOPHAGE[1]

BACTERIOPHAGE does not grow in bacterial cells suspended in water. We have found that bacteriophage will multiply in bacterial cells suspended in dilute solutions of pure glycine. The increases are from 8- to 10-fold during 3-hour incubation at 37° C. The bacteria do not multiply under these conditions in a 6.5-hour observation period.

The details of a typical experiment follow: Bacterial cells (*Es. coli*) in nutrient broth were infected with phage,[2] centrifuged down with their attached phage and resuspended in distilled water. 0.1 cc samples of this suspension were added to 0.9 cc samples of the following solutions: glycine (200 mg per cent.), nutrient broth, synthetic medium,[3] distilled water, phosphate buffer (pH 7.0) and 0.9 per cent. sodium chloride. In the nutrient broth and synthetic medium controls, phage growth (measured by plaque count) was rapid as we have described in a previous communication.[2] In the distilled water, buffer and saline controls there was no phage growth. In fact, in the water and in the saline the phage decreased. This we attribute to the death of phage-infected bacteria. In the glycine solution the phage increased, after a latent period of more than 35 minutes, to 6 times its original value at 100 minutes and 8 times the original value at 150 minutes.

The following substances were negative in this respect: asparagine, glucose, glucose in phosphate buffer, arginine, nucleic acid, alanine and diglycylglycine. The synthetic medium in which glucose and asparagine were the only organic constituents permitted phage growth, and yet these constituents separately did not support the growth of the virus.

Our sample of nucleic acid, which was probably impure, permitted the bacteria to grow slowly without accompanying phage growth. Evidently the processes within the cell which permit it to multiply are not identical with those required for phage growth, and *vice versa*.

The conditions under which the above experiments were carried out exclude the possibility of a failure of the virus to come in contact with its substrate in the bacterial cell. The failure of phage growth in distilled water and other pure solutions (*i.e.*, as distinguished from broth) is to be ascribed to the necessity of specific substrates for the growth of the phage.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

GLASS TUBES FOR REARING PHLEBOTOMUS AND OTHER INSECTS[1]

IN rearing Peruvian Phlebotomus sandflies in connection with studies on Carrión's disease, we have devised a new type of porous breeding-vessel which is also of value in the handling, transportation and rearing of other insects.

All methods of rearing sandflies in the laboratory involve keeping the engorged females at a high degree of humidity in a vessel with a moist inner surface on which the eggs may be laid. The commonest type of breeding-vessel is a small porous earthenware pot. This serves very well for mass rearing but is not convenient when large numbers of sandflies are to be handled separately. Adler and Theodor[2] secured oviposition by keeping females in glass tubes temporarily cemented to porous stone. We have endeavored to devise units complete in themselves which would combine the convenience of the glass tube with the essential functions of the porous breeding-pot, a result finally achieved by simply filling one end of the tube with plaster of Paris. Tubing with a bore of 8–9 mm is satisfactory for most sandflies, though a large species, such as *Phlebotomus peruensis*, requires larger tubing. A convenient length is eight centimeters. The plaster should extend into the tube for 10–12 mm. The open end is stoppered with cotton. These rearing-tubes may be made in quantity by standing bundles of cut tubing in dishes of freshly mixed plaster.

Before the tubes are used the plaster is moistened by contact with wet cotton. When containing sandflies they are stored, plaster end down, in moist earthen pots or in pans with a thick bottom layer of plaster. The highest degree of moisture short of condensation on the glass walls is desired. Plaster with the correct moisture content has a characteristic appearance which the operator soon learns to recognize. The eggs are laid on the plaster or on the glass just above it. The original tube may be maintained as a breeding-vessel for the larvae, but since there is usually not room enough for the progeny of one female, it is better to transfer the eggs to the standard breeding-pot. The eggs are not injured by immersion in water and transfer is easily made with a pipette and water.

[1] Supported by a grant-in-aid from Mrs. Seeley W. Mudd.

[2] E. L. Ellis and Max Delbrück, *Jour. Gen. Physiol.*, 22: 365, 1939.

[3] For the composition of this medium, see Max Delbrück, *Jour. Gen. Physiol.*, 23: 643, 1940.

[1] Contribution from Departamento de Entomología Médica, Instituto Nacional de Higiene y Salud Pública, Lima, and Department of Comparative Pathology and Tropical Medicine, Harvard Medical School, Boston.

[2] S. Adler and O. Theodor, *Proc. Roy. Soc. London*, Series B, 116: 505–515, 1935.