

GLYCINE—AN ESSENTIAL FACTOR FOR THE GROWTH OF BACTERIOPHAGE¹

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 INSECTS¹

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 Theodor² 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.

Adult sandflies of either sex live as long in the plaster-plug tubes as in the standard breeding-pots, and we believe that the average yield of eggs is greater.

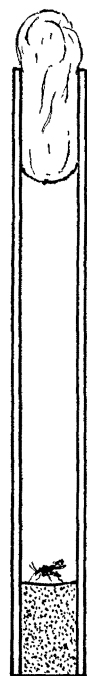


FIG. 1.

The larvae, however, get along better and with less attention in the earthen pots. Our routine rearing technique is therefore a combination of the two methods. The tubes may be autoclaved and used a number of times, but in practice it is more satisfactory to use only fresh plaster.

In addition to serving as breeding-vessels for *Phlebotomus*, the plaster-plug tubes have proved useful in a number of other ways. They permit any desired degree of moisture and thus provide an excellent container for the transportation and temporary storage of living insects. They are in routine use for catching sandflies and other insects in the verruga zone and transporting them alive to the laboratory in Lima. They may prove useful in shipping live insects considerable distances. With these tubes we have secured, through the cooperation of a physician whom we instructed in their use, living sandflies and eggs from a region several days' journey from Lima.

Tubes of this type can doubtless be adapted to the rearing of various other insects. Though we have not had occasion to try them out extensively, we have used them successfully in rearing several species of fleas.

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THE ADMINISTRATION OF DRUGS TO RATS

It is often necessary to give experimental animals quantitative doses by mouth, and frequently the material given is distasteful. Even those with much experience in giving a stomach tube to rats occasionally kill a valuable animal and at best the process is time consuming and unpleasant. A substitute technique has been found to be successful. Dissolve or suspend the material in a sucrose solution and measure it from a needleless tuberculin syringe into a rat's mouth. All rats like sugar and will take anything that can be made to taste sweet. Bile salts and other bitter material is consumed better in suspension than solution. It is recommended that two or three practice periods precede the experimental feeding. If the rat lies on its back with head slightly raised, there is no danger of spilling if the stream from the syringe is adjusted to the rate at which the rat laps. Most rats will try to turn over until this position is conditioned to the pleasure of drinking the sugar water.

This method has been used on several hundred rats, including many suffering from complete anorexia due to adrenalectomy or severe diseased conditions. Vitamins, ethynil testosterone and other hormones, sulfapyridine and many other substances have been given. The method has failed only in the administration of such irritants as CCl_4 , where the trauma to the tissues of the mouth outweighs the appeal to the "sweet tooth."

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KEEP BOTTLE-TOPS FREE FROM DUST

LABORATORY bottles invariably gather dust. After pouring from them fluid remains on the lip and around the stopper, to dry out, effloresce or otherwise create and attract dust. Cleaning them daily is a time-consuming labor, with danger of contamination from the usual wet cleaning-cloth. Such dirt and contamination may affect laboratory procedures adversely, particularly in the case of preparations for microscopic study.

A simple and effective means of keeping bottle-tops clean is to cover each with an inverted paper drinking-cup. These cups may be had in various sizes to fit different types of bottles; they are inexpensive and may be discarded when soiled.

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