with oral penicillin were also examined for inhibitory substances against this organism. Three of these showed inhibition in serum dilutions of 1:4. The greater majority of these persons had received penicillin many times in the past. The possibility that penicillin in some cases may actually induce formation of these inhibitory substances is worthy of consideration. In no instance was the penicillin concentration itself sufficiently high to act as an inhibitor of the test organism. Inasmuch as the effect of these inhibitory substances on the validity of results obtained by this serial dilution method for determining streptomycin is practically negligible, the use of a control series on each serum does not appear necessary.

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Continuous Anesthesia for Insects

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Although surgical procedures on insects have already furnished a substantial body of information with regard to developmental physiology and morphogenesis, such studies have in most cases been difficult to perform, due to the lack of an adequate method of maintaining insects anesthetized for prolonged periods of time. Many insects, especially in immature stages, have shown marked resistance to ether, the agent most commonly employed, and, furthermore, its effect is usually so transient that only the briefest procedures are generally possible before the anesthesia must be repeated.

It has long been known that insects are rapidly and reversibly anesthetized by carbon dioxide, but, here again, recovery occurs so quickly that little can be accomplished. What is needed is a method of administering continuously an anesthetic concentration of carbon dioxide.

During the past year such a method has been developed and tested. It has now been used routinely on a sufficiently large array of insect species to demonstrate its general utility, and it has also been used with uniform success at a number of other laboratories. The only requirements are a Buchner funnel of suitable size and a tank of carbon dioxide (Fig. 1). The operations are performed in the open depression of the funnel, through the bottom of which passes a slow stream of gas from the cylinder. Since carbon dioxide is heavier than air, it persists in the mouth of the funnel, making a lid unnecessary. Any depth of anesthesia can be established and maintained by merely adjusting the rate of gas flow.

For minute dissections it has been found most satisfactory to mount the funnel flush in the top of a table and just beneath a dissecting microscope. For microscopes with elevated stages, the funnel can be fitted

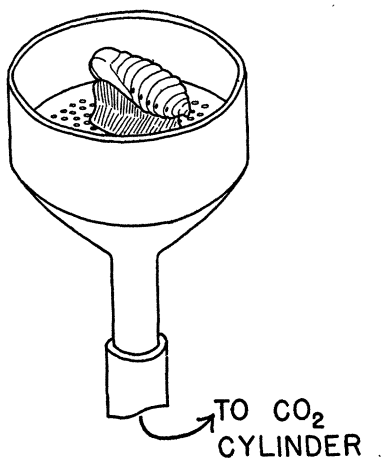

Fig. 1.

to a metal plate and substituted for the glass stage, thus affording a portable unit. In order to indicate the rate of flow of the gas and to prevent evaporation from the insect, it is desirable to bubble the carbon dioxide through water before passing it into the funnel.

The animal to be studied is simply placed in the funnel, where it rapidly becomes anesthetized. The rate with which this occurs varies enormously among different types and stages of insects, being almost instantaneous for most adult and larval forms and considerably longer for pupae. In the latter case it may be necessary to cover the funnel briefly in order to induce anesthesia. For most insects a tension of carbon dioxide amounting to one-fourth of an atmosphere serves to maintain anesthesia, so that a slow flow of gas suffices. When used routinely, a large cylinder of carbon dioxide lasts for several months.

The rate of recovery following removal from the funnel also shows great variation among different insect species, but in my experience complete recovery always occurs, even though the anesthesia may have lasted for considerably more than an hour.

Insects anesthetized by this means show complete relaxation; the integument can be opened, the operation performed, and the body wall sealed shut again without the loss of a drop of blood. The method can be confidently recommended to students of insect physiology.