Observations on the Fate of Vertebrate Erythrocytes and Hemoglobin Injected into the Blood of the American Cockroach, (*Periplaneta americana* L.)[1]

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In the process of the development of a series of methods designed to demonstrate the symptoms of malfunction of various insect organ systems, an effective method was sought to bring about the diminution of the number of phagocytic cells in the blood of the American cockroach. Previous work by Yeager *et al.* (*1*) described the injection of carbon black and trypan blue for this purpose. Their results showed that particles of the materials were engulfed by the cells, but there was no significant reduction in the total number of cells, and complete functional elimination of the cells was not claimed.

To supply a foreign body of sufficient size, with charing the blood and the introduction of this into the insect under aseptic conditions. The integument of the insect to be injected was sterilized externally by sponging the area with 70% alcohol.

There was no shock effect observed with the initial injections, but the cockroaches showed definite signs of weakness within 24 hr. Successive blood cell counts made on individuals showed a significant decrease in the number of phagocytes, and those remaining were blocked with erythrocytes and were in the process of disintegration (Fig. 1). Corresponding to the decrease in phagocytes there was always a very sharp increase in the population of a large, capsulate, rod-shaped bacterium in the blood. The injected insects died within 72 hr; death was attributed to septicemia. The injection of citrated plasma, hemoglobin in physiological saline, hemoglobin in distilled water, or laked blood with the stroma in distilled water showed none of the above effects. From this it has been concluded that vertebrate erythrocytes are very effective in blocking and destroying the phagocytic cells of this insect; that the principal function of the blood cells of the cockroach is phagocytosis; and that the regeneration of these cells is a very slow process.

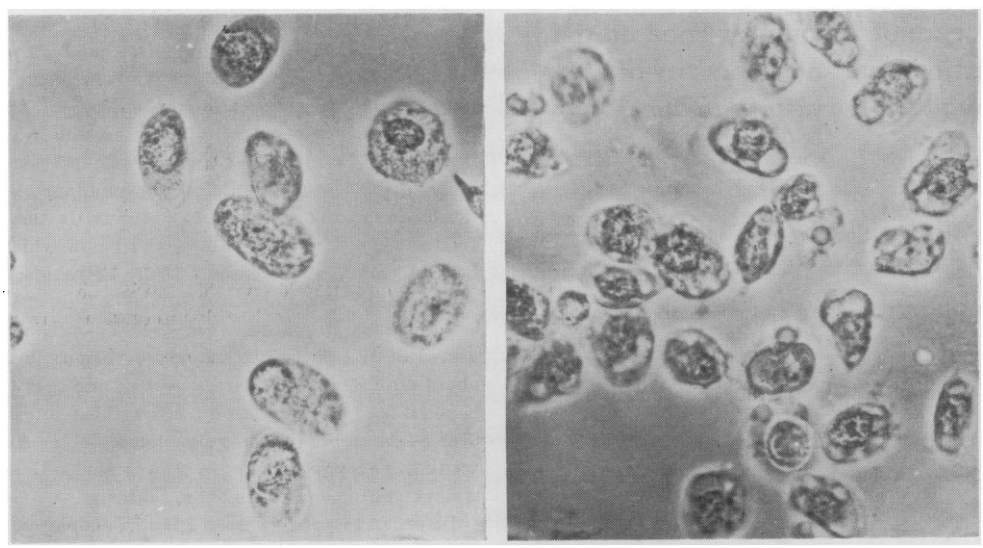

FIG. 1. Left: normal cockroach blood cells. × 600. Right: cockroach blood cells blocked with bovine erythrocytes. × 600.

acteristics that would render a complete blocking of the phagocytic cells of insect blood, vertebrate erythrocytes from the pigeon, cow, rat, and human were injected into the body cavities of cockroaches. The injections were made both as citrated whole blood and as suspensions of the erythrocytes in Ringer's solution. About 20 µl of the blood or the suspension was injected per insect. More than 200 insects were injected and observed during the course of these experiments. The technique used for the injection included the usual aseptic precautions for drawWhen hemoglobin in solution was injected, the results were quite different from those reported by Wigglesworth (*2*), who injected this material into various blood-sucking insects, including the reduviid *Rhodnius prolixus*. In the cockroach the hemoglobin is not excreted as such, since the feces gave a negative benzidine test. The red color of the blood which was imparted by the presence of hemoglobin remained for as long as 16 days with no visible diminution of intensity as observed by the filter paper spot method. Spectrophotometric determinations of samples of blood from cockroaches that had been injected for periods up to 6 hr showed absorption maximum between 500 and 600 mµ. These were identical with the absorption curves of aqueous solutions of oxyhemoglobin, a fact which indicates that no significant change in the

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chemical structure of the hemoglobin had occurred. This observation suggests the possibility of using hemoglobin as a dye for blood volume determination. Further development of this procedure will be described later.

Twenty-three days after the injection of hemoglobin, a low concentration of brown pigment was observed in the pericardial cells of some individuals. This was thought to be a bile pigment derivative such as was described by Wigglesworth (*2*), but the quantities of the material were too small for chemical determination.

The use of vertebrate erythrocytes for blocking the phagocytic cells of the cockroach, and the subsequent determination of the effects of their elimination, offer an excellent means for the comparative determination of the function of insect blood cells. Hemoglobin appears to be physiologically inert when injected into the blood of a cockroach and may offer an excellent dye for blood volume determination.

References

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The Influence of Amount of Food on the Reproduction Rate and Longevity of a Suctorian (*Tokophrya infusionum*)[1]

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At the April 1950 meeting of the Federation of American Societies for Experimental Biology, in Atlantic City, a paper was presented by A. J. Carlson and F. Hoelzel (*1*) on the effect of rich, bulky, and poor diets on fertility in rats. These authors found that fertility drops greatly if food is too rich and abundant.

I have been obtaining analogous results for over a year in studies on the feeding habits of a protozoan, *Tokophrya infusionum*. *Tokophrya* is exceptionally favorable material for feeding experiments: (1) It feeds only on living ciliates (e.g., *Tetrahymena*) which become attached to its tentacles. This makes possible the regulation of the amount of food and time of feeding. (2) Both *Tokophrya* and the ciliate may be kept in bacteria-free cultures. (3) There is no limit to the amount of food ingested, and therefore it is easy to create a state of overfeeding. (4) *Tokophrya* does not reproduce by binary fission like other protozoa but by endogenous budding, and the reproducing adult survives for a period of weeks or months. This makes it possible to perform experiments on the same individual for a considerable length of time. (5) The reproduction rate for individual embryos is a matter of hours.

The adult *Tokophrya*, in common with the majority of Suctoria, is sessile, being attached by a stalk to the substrate. It varies in size from 17 to 50 μ. *Tokophrya* reproduces by endogenous budding, forming one bud, the embryo, located within a brood pouch. This embryo, which is ciliated, rotates within the brood pouch for 10–20 min, and after that time is ejected. The liberated embryo swims for a variable length of time (several minutes to several hours). Metamorphosis then takes place. The embryo becomes attached to a solid substrate, loses its cilia, and forms a stalk and tentacles.

The largest number of embryos formed by one individual in 24 hr was found to be 12. This occurs only in well-fed individuals. *Tokophrya* starved for over 24 hr does not produce embryos. A young adult *Tokophrya* not fed after metamorphosis is able to form one embryo during its life, but does not reproduce at all if it comes from a poorly fed parent.

To find out to what extent the amount of food influences the reproduction rate, single *Tokophrya* were mounted in hanging drops in moist chambers with 1, 2, 3, or more *Tetrahymena*. Both *Tetrahymena* and *Tokophrya* were from bacteria-free cultures. After 24 hr counts were made only in those cases in which all the introduced *Tetrahymena* had become attached immediately to the tentacles. In these preliminary experiments it was found that there is an optimum in the amount of food consumed in 24 hr, above and below which the number of embryos decreases. If 1 *Tetrahymena* is consumed, only 1 embryo is formed within 24 hr. Two embryos are produced when 2–3 *Tetrahymena* are ingested in 24 hr. Only 1 embryo is formed in 24 hr when about 40 *Tetrahymena* are ingested, and 2 if the number is about 35. If *Tokophrya* feeds without interruption for over 24 hr, reproduction stops completely. A constantly feeding *Tokophrya* changes to a giant individual which is dark and opaque, with short tentacles.

In order to produce such giant *Tokophrya*, about 100 *Tetrahymena* were introduced into a hanging drop containing 1 adult well-grown *Tokophrya*. Such hanging drops were kept for 48 hr in moist chambers. Under these conditions no reproduction occurred, but the heavily feeding *Tokophrya* changed to a giant individual (180 μ in diameter in contrast to a normal average of 35 μ). The giant gradually lost its tentacles and after several hours disintegrated.

It was thought that the cessation of reproduction and the later disintegration of the giant individuals might be due to the accumulation of waste products in the medium. To rule out this possibility, the same experiments were repeated with certain modifications. Test tubes filled with autoclaved spring water were used instead of hanging drops. Both *Tetrahymena* and *Tokophrya* keep near the surface of a liquid medium; therefore it was not necessary to introduce a very large number of *Tetra-*

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