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.

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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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*hymena* to create conditions favorable for heavy feeding. Approximately the same amount of food was introduced into 8 tubes by means of a platinum loop. The 8 tubes were divided into two series. Series A contained 1–3 *Tokophrya* in each of 4 tubes. Series B contained about 40 *Tokophrya* in each of 4 tubes. In the tubes of Series A no young *Tokophrya* were found after 48 hr. There were present only giant individuals with characteristic short tentacles, and some of the individuals had already disintegrated. A large number of *Tetrahymena* available as food was still present. On the other hand, the tubes of Series B were filled with embryos and young adult *Tokophrya*, and had no traces of *Tetrahymena*. Repetition of these experiments gave the same results. After 48 hr about 30 *Tokophrya* were added to each of the tubes of Series A containing only giant *Tokophrya* and abundant food. During the next 48 hr these tubes contained embryos and healthy-looking individuals. The giant *Tokophrya* had disintegrated. These experiments indicate that amount of food, not accumulation of waste products, causes the cessation of reproduction.

Preliminary studies show also that food influences the longevity of individual *Tokophrya*. Since the species reproduces by endogenous budding and the same reproducing adult remains, the possibility exists of controlling the effect of food on the length of life of the individual. Without food an individual is able to survive for 2 weeks or more. Evidently underfeeding favors longevity in *Tokophrya*. These experiments agree with the results on underfed mice reported by Tannenbaum (*2*).

It is of great interest to note that the amount of food ingested is an important factor in the reproduction rate and longevity of individuals both in the Mammalia and in the Protozoa. Since this relationship exists in these two far-separated phylogenetic groups, it may be a fundamental and basic biological factor affecting the life of all living organisms.

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## Photoelectric Spot Analysis of Antimony and Bismuth


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The application of photometry to spot analyses was first studied by Bull (*1*) and Black (*2*). We have used a photometer having a photoelectric tube for spot analyses of antimony and bismuth ions. Two-stage amplification was used, and the intensities of light were recorded by a microammeter. Storage batteries and a small bulb were used as a source of light. A solution filter is placed before the phototube for the purpose of obtaining nearly monochromatic light. For antimony ion, potassium chromate solution was used, and for bismuth ion, copper sulfate solution gave properly monochromatic light. The reagents used and the colored products are given in Table 1.

First, a filter paper is immersed in the reagent solution; then one drop of the test solution is dropped on it, and the colored spots are obtained on the filter paper. In the case of antimony ion, the molybdenum blue does not develop well at room temperature; hence the filter paper on which the antimony test solution is dropped is put into a steam bath for about 5 min. Molybdenum blue is produced only by the trivalent ion of antimony, so the antimony solution was made carefully, and the content of trivalent antimony ion was analyzed by ordinary chemical analysis.

TABLE 1

| Elements | Reagents | Product (color) |
|---|---|---|
| Sb | $H_3PO_4 \cdot 12MoO_3 \cdot nH_2O \cdot 5\%$ sol | Molybdenum blue (blue) |
| Bi | Cinchonine KI sol* | $BiI_3 \cdot B \cdot HI$† (pink) |

* Cinchonine 1 g+; KI 2 g+; $H_2O$ 100 ml+; $HNO_3$ a few drops.
† B : organic base.

The filter paper showing a colored spot is placed between the bulb and photoelectric tube, and a screen having a small round hole (12 mm in diameter) is inserted between the bulb and the filter paper. The colored spot on the filter paper is centered on the hole, and part of the light is absorbed by the colored spot. The indicated value of the microammeter is recorded when the light transmitted through the colored spot is smaller than that through the rest of the filter paper. The value of the former is shown as $I$ and that of the latter as $I_0$. The error caused by variations of thickness of the filter paper is corrected by a blank test of each filter paper.

The results with solutions of the various concentrations of antimony and bismuth are shown in Table 2.

TABLE 2

| Content in one drop* γ | | $I/I_0$†, % | Metallic ion | Content in one drop γ | | $I/I_0$, % | Metallic ion |
|---|---|---|---|---|---|---|---|
| 0.18 | Sb | 93.8 | Sb only | 12.8 | Sb | 19.7 | Sb only |
| .30 | " | 89.0 | " " | 0.60 | Bi | 97.0 | Bi " |
| .45 | " | 88.7 | Sb and Bi | 1.48 | " | 94.9 | " " |
| .6 | " | 85.1 | Sb and Bi | 3.0 | " | 86.7 | " " |
| 0.9 | " | 75.5 | Sb only | 4.0 | " | 83.6 | Bi and Sb |
| 1.8 | " | 61.3 | " " | 4.8 | " | 81.1 | Bi and Sb |
| 3.6 | " | 43.9 | " " | 5.9 | " | 77.7 | Bi only |
| 6.4 | " | 35.1 | " " | 11.9 | " | 69.9 | " " |
| 9.0 | " | 23.9 | " " | 29.7 | " | 54.0 | " " |

* The volume of one drop is 0.032 ml.
† Mean value of 3 determinations.

Interference between Bi and Sb was not noticeable. The writer applied this method to an analysis of Bi ~ Sb