DIGESTION IN BLOWFLY LARVAE, PHORMIA REGINA MEIGEN, USED IN THE TREATMENT OF OSTEOMYELITIS.*

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The role of digestion in blowfly larvae is closely associated with the use of these maggots in the treatment of osteomyelitis. It is interrelated with such factors as methods of aseptic rearing, food requirements, and the activities of these maggots within the wounds involving the success of the treatment. In this study emphasis was placed on the qualitative determination of enzymes present and their location in the digestive tract, the effect of bacteria on digestion, and the analysis of the excreta.

Many articles have been written about digestion in various flies, most of the investigators attempting to prove or disprove the necessity for the presence of bacteria in the food. Atkin and Bacot (1917) maintained that bacteria or yeast was necessary in the food of the mosquito *Stegomyia fasciata.* Baumberger (1919) showed that Drosophila could not be raised without live yeast. According to Bogdanow (1906 and 1908) Calliphorid larvae failed to develop in the absence of micro-organisms. Guyenot (1906, 1907, and 1917) stated that Lucilia lived on the liquid products of bacteria, were unable to produce digestive ferments, and that this was done for them by bacteria. On the other hand, Wollman (1911) maintained that Calliphorid larvae could be raised without the presence of bacteria. Glaser (1924) bred two generations of Drosophila on aseptic yeast, and Northrup (1926) succeeded in rearing 230 generations of Drosophila aseptically. Hobson (1932a) raised larvae of *Lucilia sericata* aseptically on autoclaved sheep's brain, but was not successful when he used types of muscle such as that found in a guinea pig or a cod (1932b).

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A few articles have been written concerning the digestive enzymes present in various flies. Bogdanow (1928) succeeded in breeding eleven continuous generations of *Calliphora erythrocephala* on human excreta and concluded that this fly built up proteins from simple nitrogenous compounds and amino acids. Trypsin was found in the excreta of Calliphorid larvae according to Weinland (1906). Protease, amylase and erepsin in Calliphorid and Lucilia larvae were found by Wollman (1922). Probably the most complete work on digestion of flies was done on *Lucilia sericata* by Hobson (1931), who found traces of amylase in the salivary glands while tryptase, peptidase and lipase were found in the midgut. Proteolytic enzymes were also found in the excreta.

PREPARATION OF MATERIALS FOR ENZYME TESTS.

Full grown *Phormia regina* larvae which had not yet ceased to feed were used throughout the tests. They were starved until their alimentary tracts were empty by placing them in a specially prepared filter that provided for a slow stream of water to pass over them. This procedure, similar to that devised by Hobson, not only kept the larvae moist during starvation, but it also prevented the ingestion of excreta. The alimentary canals of larvae treated in this manner were usually perfectly clean within 6 to 8 hours, and by this method we were certain that any positive tests obtained would be from enzymes located in the tissues of the larvae.

The digestive tracts were then removed from the larvae, after which glycerol extracts were made separately of the salivary glands, crops, anterior-middle- and posterior-portions of the mid-intestines, and hind intestines. The organs, as well as the division of the mid-intestine into three parts, are shown in Figure 1, which illustrates the gross anatomy of *Phormia regina*, having a digestive tract essentially the same as that of *Calliphora erythrocephala* (Lowne, 1890) and *Lucilia sericata* (Hobson, 1931).

When fifty larvae were so dissected each of the various portions was completely macerated in a mortar and stored in vaccine tubes under toluene.

Preparations from the excreta were made by placing one hundred washed larvae in the filter, and allowing 50 cubic centimeters of water to drip over and through them for four hours. This solution was then stored in a similar manner by

placing it in vaccine tubes under toluene. The possibility of regurgitation was not eliminated by this procedure, but this was not essential since we desired to determine all the digestive enzymes emitted by the larvae, as they would all be present in the treatment of a wound.

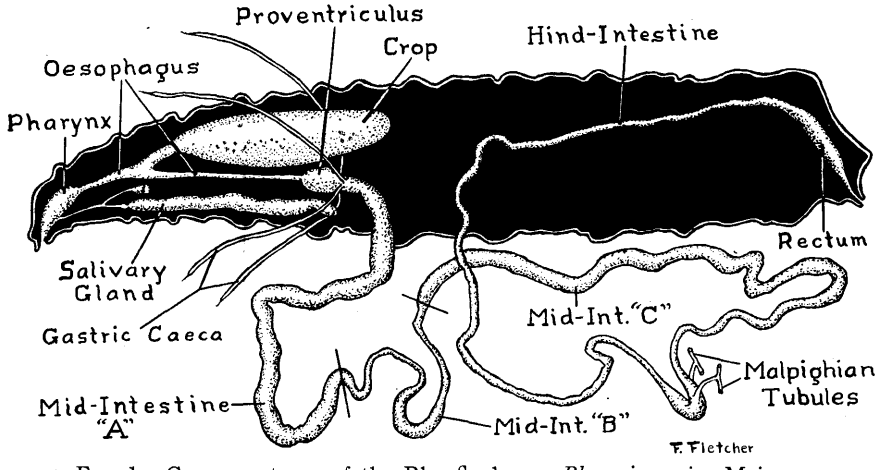

FIG. 1. Gross anatomy of the Blowfly larva, *Phormia regina* Meigen.

DETECTION OF ENZYMES AND HYDROGEN-ION CONCENTRATION.

The methods of enzyme analysis were essentially those devised by Swingle (1928) with certain modifications being made according to Wigglesworth (1927, 1928 and 1929) and Hobson (1931). A very brief summary of the methods used were as follows:

Amylase was tested for by glycerine extracts acting upon .5 cc. of 0.5 per cent starch solution, potassium iodide added after 72 hours incubation at room temperature. The Flückiger test was used to detect the presence of reducing sugars. *Maltase* was tested for by adding glycerine extracts to a 15 per cent solution of maltose, incubated for 72 hours, and the osazone test used to determine the presence of glucose. The test for *Invertase* was the addition of the glycerine extract to 0.5 cc. of 15 per cent solution of sucrose, and, after 72 hours incubation, the presence of fructose and glucose tested by using the osazone test and the presence of reducing sugars by the Fluckiger test. The test for *Lactase* was the same as that for maltase with the exception of using a 3 per cent lactose solution as the

substratum. *Lipase* was tested for by using the Brom Thymol Blue Emulsion test. An emulsion of olive oil and acacia was added to the glycerine extract in which the presence of Brom Thymol Blue and lipase would cause a color change from blue to greenish-yellow after 96 hours incubation. *Trypsin* and *Pepsin* were tested for by using the blood fibrin test as outlined by Swingle, the enzymic action breaking down the fibrin previously stained with Ruthenium Red for the trypsin test, and stained with Amaranth for the pepsin test. *Erepsin* was tested for by using the Modified Sorensen Method as outlined by Swingle.

TABLE I.

Digestive Enzymes in Contaminated Larvae of *Phormia regina* Meigen.

Enzyme	Salivary Glands	Crop	Anterior Mid-gut	Middle Mid-gut	Posterior Mid-gut	Hind Gut	Excreta
Amylase	±	—	—	—	—	—	—
Maltase	—	—	—	—	—	—	—
Invertase	—	—	+	—	—	—	—
Lactase	—	—	—	—	—	—	—
Lipase	—	—	±	—	±	±	±
Trypsin	—	—	+	—	+	+	+
Pepsin	—	—	—	—	—	—	—
Erepsin	—	—	+	—	+	±	±
pH	7.18	7.24	7.15	6.0	7.18	7.54	7.1

The hydrogen-ion concentration was determined in the various portions of the digestive tract as well as the excreta. The fluids from the tracts of freshly-killed larvae were taken up in small capillary tubes, indicators added and the resulting colors compared with a similar set of capillary tubes made up with standard indicators. This method was checked by using a standard micro-comparator set.

The results obtained are indicated in Table I. The plus sign (+) represents a relatively strong reaction, while the negative sign (—) indicates no reaction, and the two signs together (±) point out a weak reaction. These tests were repeated in three different sets of experiments and only once was the presence of *invertase* indicated.

EFFECT OF BACTERIA ON DIGESTION.

A technique was devised for the rearing of aseptic larvae. This was thought advisable for two reasons: first, that it offered a check on controversial results of former investigations, and second, that it provided a means of proving that whatever digestion occurred was due to enzymes present and not to bacteria. Fresh lean beef was ground up and weighed into 12-gram amounts and placed in large test tubes that had been previously autoclaved. This set-up was then heated in a water bath at about 75° C. for 30 minutes on four successive days, thereby killing off the vegetative stages of any bacteria that might have developed after the first and second heatings.

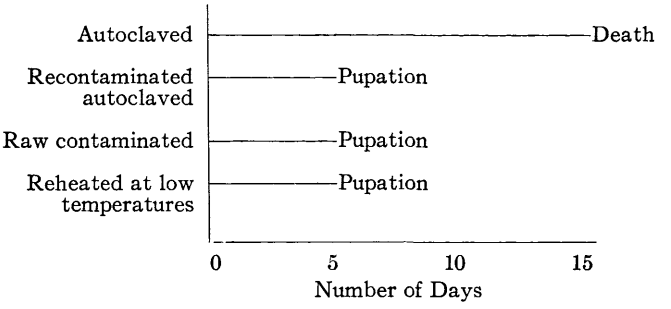

FIG. 2. Rate of development of larvae on varied treatments of the same medium.

After this part of the procedure was finished eggs of *Phormia regina* were implanted that had been previously sterilized by washing them in an alcoholic solution of 1 : 2000 mercuric chloride, about 30 eggs placed in each tube. Check tubes on rate of development were made by placing raw contaminated beef in one tube, recontaminated autoclaved beef in the second, and sterile autoclaved beef in the third. The larvae were allowed to grow without any disturbance of the sterile cotton plugs that closed the test tubes. After the larvae had become full grown, the contents of the test tubes were examined for bacterial contamination by implanting small portions of them in anerobic and aerobic broth tubes, on agar plates, and by stained smears on microscope slides.

The maggots in those tubes that proved to be sterile were then divided into two lots. The larvae of the first lot were allowed to continue their development, and the flies that

emerged were full size and seemed normal in every respect. Some rather significant results were obtained when the rate of development of these larvae were compared with those reared in the check tubes. Figure II illustrates these results graphically, carried only through the feeding period (Haub and Miller, 1932).

At a constant temperature of 29° C., and a relative humidity of 70 per cent, maggots grown in the media made sterile by this low heating method pupated on the fifth day. The same rate of development occurred in those tubes containing raw contaminated beef and recontaminated autoclaved meat. Those maggots grown in the sterile autoclaved meat did not mature at all, but remained for over two weeks in a size corresponding to the second or third instar, and at the end of that time died. This experiment was repeated twice and identical results occurred.

This would seem to indicate that some food factor is destroyed when the meat was autoclaved, that this factor is somehow replaced when this same autoclaved food is contaminated (Glaser, 1924), and that sterilizing at lower temperatures does not destroy this factor at all.

The second lot of maggots, reared by this method was then set aside to be used as a check on the enzymic reactions obtained from the larvae raised on natural contaminated meat as summarized in Table I. The same methods were used for the determination of enzymes as were used previously, except that the maggots were handled under the most aseptic conditions possible. All apparatus used was sterilized. Each maggot was dissected under sterile water, and the instruments used were sterilized in mercuric chloride after each dissection. The portions of the digestive tracts obtained in this manner were then placed in their respective vaccine tubes that had been previously autoclaved, and after the proper chemicals had been added to determine the enzymic reactions, each tube was capped with toluene.

By using these precautions, we were then positive that whatever digestive reactions occurred were due to the presence of enzymes and not to any bacteria that otherwise would have been present.

Hobson (1932a) had previously checked only for the presence of tryptase and pH values in *Lucilia sericata*, and found that tryptase was present in aseptic larvae and that the gut reactions

in them were normal, when he reared the larvae on autoclaved brain mush. Our experiments included the testing for all the enzymes that had been found present or absent in the contaminated larvae, and the aseptic larvae were raised on lean beef. The results obtained are summarized in Table II.

A comparison of the results obtained from those maggots raised in a contaminated medium and those reared aseptically showed that the only difference occurring was a positive test for invertase in the anterior portion of the mid-guts of the contaminated larvae. Furthermore, since the reaction for the

TABLE II.

Digestive Enzymes in Aseptically-reared Larvae of *Phormia regina* Meigen.

Enzyme	Salivary Glands	Crop	Anterior Mid-gut	Middle Mid-gut	Posterior Mid-gut	Hind Gut	Excreta
Amylase	±	—	—	—	—	—	—
Maltase	—	—	—	—	—	—	—
Invertase	—	—	—	—	—	—	—
Lactase	—	—	—	—	—	—	—
Lipase	—	—	±	—	±	±	±
Trypsin	—	—	+	—	+	+	+
Pepsin	—	—	—	—	—	—	—
Erepsin	—	—	+	—	+	±	±
pH	7.0	7.0	6.9	6.6	6.9	7.2	6.8

presence of invertase occurred only once in the three times that the experiment was repeated, it suggests that the hydrolysis of sucrose was not caused by the enzyme but probably by bacteria.

SUMMARY.

In the treatment of osteomyelitis, the role of digestion in blowfly larvae is a significant one, being interrelated with the success of the treatment. A study was made to determine enzymes present, the effect of bacteria on digestion, and the analysis of the excreta.

The only enzymes found present in various portions of the digestive tract were amylase, lipase, trypsin and erepsin.

A method was devised to raise flies from egg to adult aseptically by repeated heatings of the media at low temperatures. It was demonstrated that some food factor is destroyed when the media was autoclaved, that this factor is somehow replaced when this same autoclaved media is recontaminated, and that sterilizing at lower temperatures does not destroy this factor at all.

By this method a check was provided on enzymic determinations in contaminated larvae, and the only significant difference obtained was in the test for invertase; it was found present in the contaminated larvae but not in the larvae reared aseptically, which would seem to indicate that the hydrolysis of sucrose was not caused by the enzyme but probably by bacteria.

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Man and Metals.

This is the story of man's advances as viewed from the standpoint of mining and metals. From the "Age without Metals" to our modern times the author has given us an insight into the various aspects of mining and metals, and how they have tended to shape our civilization. He considers the use of metals and manners of obtaining them from earliest times through the Mediterranean civilizations, the Medieval developments of the Old World, then in the New World starting with the Conquest of Peru and coming down to the mining in Australia, and finally to Cecil Rhodes and Southern Africa. Coal and iron are considered as separate from the rest.

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The work is well written and very readable, sustaining interest throughout its many pages. The author is to be congratulated on the book, the entire make-up of which is pleasing.—W. BERRY.

Man and Metals, by T. A. Rickard. Two volumes, 1061 pp., 108 ill. New York, Whittlesey House, (McGraw-Hill Book Co.), 1932. $10.00.

Earth History.

This volume on geology is the first of the Century Earth Science Series and handles Earth History in an entirely new manner. There are four main sections; in the first are considered various physical processes, the time scale and cycles of earth history. The second takes up the physical history of the continents. The third considers the biologic history, and the last, "Man and Earth History."

The arrangement and manner of presentation are new and unusual in what might be called introductory geology. The style is good and the text is not burdened with technical terms or involved explanations. For the general reader it is far ahead of the general run of introductory geologies, but I question its use as a text in introductory geology, as it does not seem to be exactly "teachable." As supplementary reading or general reference it is excellent.

The book is well gotten up and is well handled by the publishers. It should find a ready place in general reading and as a supplementary text.

WILLARD BERRY.

Earth History, by L. C. Snyder. 675 pp. New York, The Century Co., 1932.