THE SECRETION OF COLLAGENASE BY MAGGOTS AND ITS IMPLICATION*

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THE SEARCH FOR an enzyme which would digest collagen led us to the conclusion that *Cl. histolyticum* was one of the most potent sources of such an enzyme, collagenase. Since this organism lives in the soil as a normal inhabitant in an anerobic state, it was felt the function of this enzyme was probably the solubilization of long chain insoluble polymers, so that in turn the organism could digest the protein breakdown products for nutritive purposes.[8] It therefore occurred to one of us (NAW) that the remarkable action of maggots in the treatment of osteomyelitis and necrotic wounds, so popular in the mid-thirties, was due possibly to the same action.

When Baer initiated the widespread use of maggots,[1] he considered their action as that of scavengers, sucking up and consuming dead tissue and bacteria. He stated that maggots destroyed only dead tissue or pathologic tissue, but he suggested that some other biological reaction probably occurred, the nature of which he did not know. Some individuals tried to explain the action of maggots through chemical means, and one of the most popular concepts was that allantoin was this agent and was excreted as a product of purine metabolism.[7, 11] In 1931 Hobson,[4] by placing washed larvae in a filter and allowing water to drip slowly through the filter for several hours, stated that he found a substance which digested collagen; he believed that it was secreted in the midgut of the larvae. This was never confirmed, however. The most recent work by Messer and McClellan in 1935,[9] who attempted to obtain such an enzyme from maggots in somewhat similar fashion, was unsuccessful. If Hobson was correct, then why did not the larvae attack healthy tissue, inasmuch as from our own experiments it had been demonstrated that collagenase not infrequently dissolved living healthy tissue?[8] It remained for Stewart[13] to show that larvae preferred necrotic tissue over normal healthy tissue, provided it was immediately accessible. However, they would attack healthy tissue.

TECHNIC

Our first attempt to obtain this enzyme by maceration of the maggots was as unsuccessful as it had been by others.[10] However, realizing that *Cl. histolyticum* produces great quantities of collagenase by action on a proper medium, it was decided to reduplicate this process with the maggots.

The species of fly employed was *Phaenicia sericata* Meigen. The original culture was obtained by placing meat in shallow containers in the open air and allowing flies to oviposit on the meat. During larval development and after the flies had reached the adult stage, they were identified according to the descriptions of Knipling[6] and Hall.[3] The particular species desired was isolated and a brood culture was main-

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tained. Water and granulated sugar were available to the adult flies at all times, and meat (beef steak or beef liver) was supplied intermittently.

When eggs were desired, meat was placed in the cage and the flies were permitted to oviposit for two or three hours. The eggs were then removed from the meat, separated, and sterilized according to the methods described by Simmons.[12] Essentially, this consisted of spreading the eggs, by means of a small spatula, on a wet, fine mesh cloth on a wet cotton pad in a petri dish. They were then immersed in a test tube containing 5 per cent formalin and 1 per cent sodium hydroxide for 10 minutes. From this point on, all materials that were used were first autoclaved and the eggs and larvae were maintained under sterile conditions.

When the sterilization was completed, a portion of the sterilizing medium was decanted and the remainder, with the eggs, was poured on a piece of surgical gauze in a Gooch crucible fixed in the neck of a specimen bottle. The crucible and neck of the bottle were covered with a glass cap to maintain the sterility of the eggs. About 50 ml. of sterile water were poured slowly over the eggs to remove the disinfectant.

The eggs on the gauze were then transferred to an Erlenmeyer flask containing 500 Gm. of autoclaved cubes of beef steak and 25 ml. of water. The flask was covered up to the neck with a heavy cloth and a light was applied to the neck. The heat from the light maintained the temperature of the culture between 35 to 40 degrees C. Maggots are very sensitive to moisture,[2] and the heat of the light also kept the neck warm enough to prevent escape of the maggots.

The larvae hatched out in 10 to 15 hours and were allowed to feed on the meat until the end of the second instar, which was approximately three days in these experiments. The maggots, water and meat were then poured into a Buechner funnel, covered with a glass plate, and filtered under suction. The filtrate was transferred to a dialysis bag and dialyzed against sterile distilled water, with gentle agitation, for eight hours.

Tests for sterility were carried out during the entire investigation from the time the eggs were first sterilized until the final check of the filtrate for the presence of collagenase. Cultures in nutrient broth and deep meat were used and all were negative. The hydrogen ion concentrations of the water and meat were checked at intervals and were found to range from 6.0 to 6.5 when the eggs were first placed in the flask to 8.0 to 8.5 when the filtrate was obtained.

When pure collagen was placed in a test tube with some of the dialyzed filtrate, digestion of the collagen occurred. Collagenase activity was assayed by using the technic previously described.[8] The crude filtrate contained 6.6 mg. of protein per ml. One milliliter of this filtrate digested 50 mg. of collagen at 37 degrees C. in 30 hours. This figure is somewhat lower than the quantity of collagenase ordinarily found in a similar volume of crude *Cl. histolyticum* filtrate.

DISCUSSION

This study essentially confirms the report originally made by Hobson in 1931. The latter found other enzymes in the midgut of the larvae, tryptase, peptidase and lipase,[5] which undoubtedly enable the digestion of the broken down molecules of protein previously acted upon by the collagenase. It appears that the maggot derives at least a portion of its nutrition in the same way as does the *Cl. histolyticum*, and in the same fashion it enabled such remarkable effects to result from maggot therapy.

SUMMARY

1. The maggot of the blow-fly, *Phaenicia sericata* secretes collagenase.

2. It is postulated that its action is to create breakdown of the long chain polymers to enable digestion to take place in the midgut of the larvae by other enzymes.

3. It is probable that the therapeutic activity of the maggot in cleansing wounds and in the treatment of osteomyelitis was in part due to the secretion of this enzyme.

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