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THE TREATMENT OF OSTEOMYELITIS (INFECTION OF BONE) WITH FLY LARVÆ.*

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AND
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The disease known to medicine as osteomyelitis is an infection of the bone and adjacent tissues which frequently becomes chronic, often results in crippling deformities and sometimes proves fatal. The usual method of treatment is surgical, involving the removal of dead bone and the attempted elimination of infected areas. Because of the site and nature of the involvement, surgical treatment alone often fails and repeated operations are frequently required.

In 1917, during the World War, the late Dr. Wm. S. Baer of Baltimore, Md., serving with the American Army in France, noted that some of the soldiers brought to the hospital from the battle field with compound fractures of the thigh, had wounds heavily infested with maggots. Contrary to expectation, their general condition and that of the wounds seemed markedly better in contrast with others not showing such infestation. Furthermore, those with maggots seemed to recover in a shorter time (Baer, 1930). With these observations in mind, Dr. Baer returned to America and some ten years later decided to try the introduction of maggots into civil practice as a treatment in stubborn cases of osteomyelitis. His clinical studies were carried on for the most part at the Children's Hospital School in Baltimore.

The first treatments were made with maggots secured from natural sources. However, it soon became apparent that this was attended by certain dangers, inasmuch as fly larvæ in

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nature live along with microorganisms that are pathogenic to man. Therefore, the direct implantation of maggots by the surgeon in an open wound would be justified only after two important questions had been satisfactorily answered. (1) Do maggots assist in the process of healing? (2) Can they be released for activity without danger of inciting further disease complications? The first of these questions was at least suggestively answered in the affirmative by Dr. Baer's observations during the war and his preliminary peacetime studies. It then became necessary to seek a satisfactory answer to the second question. The rearing of bacterially sterile maggots is but one of the many problems which have been under study during recent months in several centers, notably at the Children's Hospital School in Baltimore, the United States Naval Medical School in Washington, D. C., and the Ohio State University, Columbus, Ohio.

A COOPERATIVE PROJECT.

For many years cultures of blowflies of various types have been grown in the Department of Zoology and Entomology at the Ohio State University for purposes of studying insect physiology, behavior and rearing methods. During the summer of 1930 one of us (D. F. M.) worked with the United States Bureau of Entomology at Washington, D. C., upon the problem of rearing flies for clinical use. Since October of that year the study has been continued at the Ohio State University as a cooperative project between the Department of Zoology and Entomology, the Department of Medical and Surgical Research, and the Department of Orthopedics. This arrangement makes possible a complete series of studies beginning with the rearing of the flies and ending with the treatment of selected patients in the hospital.

REARING OF FLIES.

Several articles giving methods of maintaining cultures of flies have appeared recently, particularly those of Elizabeth K. Elgin at the Children's Hospital School (Baer, 1930–1931) and that from the Naval Medical School by Murdock and Smart (1931). While the methods employed and described by the above writers show the feasability of cultivation of larvæ for clinical purposes, much is yet to be learned concerning the factors of food, temperature, humidity, light and efficiency in

handling. These problems have been and are now under study at the University and the following information is a preliminary report of our experiences and results during the past year.

Our work has been restricted largely to the two species of blowflies *Lucilia seracata* and *Phormia regina*. These are two of our more common blowflies, easily obtained, easily reared in captivity and are the two that have been most generally used so far in the clinical work of others.

Cages.—The size and shape of culture cages is largely a matter of convenience. We use cages of about one cubic foot capacity because of ease in handling and cleaning. In cages of this size it has been found that a population in excess of 200 to 250 flies gives signs of overcrowding. Gauze or cheese cloth is to be preferred to wire screen as a covering for the cage. The flies in screen cages are more easily disturbed by objects moving near the cage, such excitement resulting in broken wings and a decrease in egg laying. Also gauze is easily replaced when soiled.

Cabinets.—Special cabinets in which the temperature and humidity may be varied permit control of oviposition, which may be desirable as will be brought out later. However, in laboratories or rooms where the temperature does not fall below 18° C. nor rise above 32° C. cabinets are not essential and our results under these conditions have been quite satisfactory. If plenty of fresh drinking water is supplied at all times the relative humidity of the air is not important as long as it does not fall below 35 per cent nor rise above 85 per cent.

Foods.—Murdoch and Smart (1931) and Elgin (Baer, 1930–1931) have discussed their methods and suitable foods in recent publications, and we have also experimented considerably in this field. After testing a variety of natural foods and a number of synthetic ones we have reached the conclusion that, if plenty of drinking water is supplied by inverting a small beaker upon a circle of filter paper in a petri dish, the only other food requirements may be adequately met with dry granulated sugar and fresh lean meat. This makes a decidedly simple diet which can be readily handled and has given very satisfactory results over a period during which a large number of consecutive generations have been reared. Elimination of liquid foods and syrups lessens the mortality from drowning and from poisons due to fermentation. The dry sugar can be

eaten readily by the flies and is kept in the cages at all times except when the flies are ovipositing. Meat should be fed at least once in each two days. It may be fed each day if the eggs laid can be used as frequently as that.

In a series of experiments on foods for larvæ, conducted under conditions of controlled temperature and humidity, the following foods were used: (1) lean beef, (2) lean beef and beef fat, (3) lean beef and trypsin, (4) lean pork, (5) beef liver, (6) fish, (7) boiled egg, (8) dried blood and casein mixed with water. Numbers 1, 2 and 4 gave the most rapid rate of development and seemed best in all other respects although 6 and 8 were utilized almost as readily by the larvæ. The life cycle was completed on all of the foods and there was no noticeable effect on the sex ratio.

Not infrequently larvæ leave their food supply before they have fully developed. Wardle (1930) states that relative humidity of the atmosphere is of considerable importance as a causative factor and that if it falls below 60 per cent the larvæ will leave the meat. We have not found this to be true if the meat does not become too dry. However, there are two factors which do play an important part in producing this reaction: overcrowding of the food supply, and, probably of primary importance, the accumulation of gases in the container used for rearing. Experiments run with a ventilator system for some of the containers and without for others demonstrated this fact.

Pupation Jars.—When larvæ are full grown they leave the food. If just prior to this the culture vessel in which they are feeding is placed in a larger container with strips of crumpled paper toweling, the migrating larvæ will crawl through the paper thus drying and cleaning themselves. They may be left here to pupate, or better, they may be removed and scattered through cheese cloth in another container. The larvæ will work their way into the folds of the cloth and there come to rest. This prevents their collecting in large writhing masses thus disturbing each other continually and probably delaying pupation. The cloth is also decidedly easier to handle than soil or sand, the natural repositories for pupæ. When pupation is complete the cloth is spread out and the pupæ removed and placed in petri dishes until emergence starts, after which they are transferred to the cages.

Segregation of the pupæ and the sex ratio.—Some workers have attached considerable importance to the matter of sex ratio in the culture cages. This is not of sufficient importance to justify the amount of labor required to count out the sexes for each cage. If a large stock of prepupæ are obtained and the first pupæ that form removed, they will, upon emergence, be found to have yielded more males than females. Likewise, the last ones to pupate will yield more females than males. If, however, all are permitted to pupate before being counted out into whatever numbers are desired for cage lots, the lots upon emergence will be found to show a sex ratio of approximately one to one. A preponderance of males is not undesirable as careful counts have shown that the males die in larger numbers during the first two weeks of a cage history.

Temperature and Oviposition.—The entire procedure of fly rearing centers around oviposition since upon this depends the supply of larvæ for the clinic. For this reason, we have paid more critical attention to this than to any other one problem. It has been studied for more than a year and over many generations. For this work we have used mostly *Phormia regina.* While light and humidity exert a minor influence, the controlling factor is unquestionably temperature. These flies have an oviposition range of from 20° C. to about 34° C. with an optimum near 26° C. At the lower temperatures, the longevity of the flies is greatly increased but the number of *eggs per female per day* is greatly decreased. At the higher temperatures, the number of *eggs per female per day* is increased but the total number of *egg-laying days* is decreased because the flies do not live so long. These two factors tend to equalize each other at the extremes but a temperature near 26° C. gives the *greatest total deposition of eggs per female.*

The practical application of these studies is self evident. If the clinical material makes it necessary to speed up the production of eggs it can be accomplished within two days by increasing the temperature in the cages to approximately 30° C. and supplying plenty of food and drinking water. However, it must be remembered that the flies will not live so long at this higher temperature and will have to be replaced at an earlier date. Consequently, the total number of eggs from any one cage will be reduced. This type of behavior in oviposition conforms to that worked out for a number of other insects that have been studied.

STORAGE.

Another problem of practical importance is the possibility of maintaining reserve stocks for quickly increasing the number of adults producing eggs, or for safeguarding against any sudden mortality thus endangering the supply of sterile larvæ for the clinic. Cold storage has been resorted to for this purpose.

The mortality of eggs in storage is too great after seventy-two hours to be of much practical application. Larvæ of various ages show the following results. Newly hatched larvæ cannot be held, to advantage, much more than one week. Larvæ, which have fed for twenty-four to thirty-six hours, will continue to feed, if returned to the food, after two or three weeks in storage. Some have been held for four weeks in refrigeration but the mortality is high. Larvæ that were full grown and ready to migrate from the food were held four weeks after which they pupated and emerged 88 per cent. The temperature used was 8° C. to 10° C. Longer periods of storage ranging from six weeks to six months, over a wide range of temperatures, gave results with a mortality so high that they were of no value. These observations offer obvious possibilities.

The storage of pupæ and adults has been tried with some success. They can both be held for one, two, or three weeks. However, at present, the storage of larvæ seems to offer the greatest possibilities.

PRODUCTION OF STERILE LARVÆ FOR THE CLINIC.

From the point of view of the laboratory, the chief objective is the production of sterile larvæ for the clinic. This part of the work is of such great importance that the utmost precaution must be followed in its pursuit. The eggs are collected from slices of fresh beef that have been placed on circles of filter paper in petri dishes. The dishes and paper have been previously autoclaved to reduce the possibility of contamination. Such preliminary precautions have shown that they contribute materially to success in the sterilization of the eggs. With flamed forceps the eggs are removed from the meat, placed in sterile vials and labeled. Within twenty-four hours they are subjected to a sterilization procedure which does not materially affect the hatching but which does eliminate the ordinary bacterial contaminants when the precautions already outlined are scrupulously adhered to. The technic employed at present

for planting the eggs is essentially that worked out in co-operation with Dr. G. F. White of the United States Bureau of Entomology at Washington, D. C. It consists of dissolving the egg masses apart by shaking not too vigorously in a vial of sterile saline with glass beads. The saline is then pipetted off and replaced by a solution of bichloride of mercury (1:2000) in 25 per cent ethyl alcohol in which the eggs are bathed for not less than fifteen minutes nor more than twenty minutes. They are then poured through a previously sterilized unit consisting of a Gooch crucible containing a small circle of cheese cloth which strains out the eggs. After washing thoroughly with sterile saline to remove any excess of the sterilizing solution the cloth containing the eggs is removed with sterile forceps and placed in a sterile tube of autoclaved agar containing a small amount of meat, much as any bacterial culture might be made. Some of the eggs and the tubes of young larvæ are tested for at least three days for aerobic and anaerobic bacteria before they are released to the hospital for use in clinical cases.

The possibility of retarding or suspending the metabolic processes in the larval stage has been mentioned above as a solution to the problem of maintaining a sufficient reserve of potential flies. This temperature control of larval development has been utilized also in the establishment and maintenance of reserves of larvæ for clinical use. Newly hatched larvæ are too small or too young as a rule to survive immediate implantation in a wound. Therefore, a certain minimum size for wound implantation which is attained in from twenty-four to thirty-six hours at 37° C. or in 72 hours at room temperature (24° C.) is desirable. When the optimum size of the larvæ has been reached, it is possible to suspend growth and development temporarily by reducing the temperature to 8° to 10° C. If the larvæ introduced into a wound are within the seventy-two hour (24° C.) growth size (even though kept for from fourteen to eighteen days at this stage by low temperature) a period of from four to five days of scavanger activity may be anticipated before pupation occurs and a new implantation is required.

CLINICAL RESULTS.

It must be emphasized that the use of fly larvæ in osteomyelitis in no sense eliminates the surgical aspects of the treatment, but is advanced as a method of after treatment

superior to those formerly advocated. The success of any form of treatment in the disease is conditioned very largely by two factors: (1) the age of the patient, and (2) the duration of the disease. Acute or chronic osteomyelitis seems to heal more readily in the very young patients, and the most resistant and difficult cases to treat satisfactorily are those of many years standing in elderly patients. Thus, the importance of an effective treatment early, that the changes which come subsequent to repeated relapses and operations may not jeopardize the possibility or permanent cure.

To Baer's 89 reported cases may be added 17 which have had more or less intensive maggot treatment following operation in the osteomyelitis clinic of the Ohio State University during recent months. In 12 of these cases healing occurred promptly in from four to nine weeks. The remaining 5 represent the first cases in our series and did not have the full benefit of the treatment because of certain limitations then existing in technic and experience. It must be pointed out that success depends in great measure on a certain acquired experience both in the establishment of an efficient laboratory routine and in the effective handling of the treatment in the patient by the surgeon. More complete data on the cases treated in this study are being presented in another communication (Wilson, Doan and Miller, 1932), together with the technic of applying and "caging" the larvæ within the infected areas of bone and tissue destruction.

The mechanism by which larval infestation assists nature in the elimination of infection and the promotion of healing is, of course, one of the chief objectives of this joint study. Through the interest and cooperation of Professor Wm. A. Starin, of the Department of Bacteriology, it has been determined from cultures of larvæ, taken from our stocks for clinical use, that no bacteriophage principal has been present, which, if introduced into the lesions inadvertently with the maggots, might have exerted a lytic effect upon the staphylococci, which have been found almost uniformly to be the causative agent in this series of cases. We have observed a marked change in the hydrogen ion concentration of the wound from an acid to an alkaline reaction following maggot implantation. Does this inhibit pathogenic bacteria? Do the enzymes or excretions of the larvæ exert any direct effect upon the healing process? Or, is it a vital phenomenon wholly related to the

scavenger propensities of this organism for dead tissue? The more or less empiric observations of clinical healing will not suffice indefinitely. An answer to these various questions must be sought for as their satisfactory understanding is essential to the scientific approach to that larger problem of wound healing and tissue repair, toward which the future work of this group is being directed and to which we feel the use of fly maggots in osteomyelitis may contribute.

The osteomyelitis clinic, which is under the direction of the College of Medicine at both the University and Children's Hospitals, is to be continued through the coming year and is available to the sufferers from this malady from anywhere in the state or surrounding community.

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