THE CULTURE OF FLY LARVAE FOR USE IN MAGGOT THERAPY

by

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I. INTRODUCTION

The therapeutic use of blowfly larvae in the treatment of osteomyelitis and certain other suppurative infections is one of the most spectacular developments in modern medicine and biology. As has been aptly said, (1) "Certainly this story of the practical application of a wartime observation will go down in medical history as one of its most fascinating chapters. Fascinating to the layman because it conceives of the hitherto repulsive fly maggot as being beneficial rather than detrimental to mankind. And fascinating to the medical profession because it invokes an entirely new principle in asepsis—a biological in contrast to a chemical antiseptic—a parasite upon a parasite, if you will."

The introduction of this new therapy to the medical profession in 1928, by the late Dr. William S. Baer of the Johns Hopkins University Medical School immediately opened many new and fascinating fields of research. Not only was the medical profession concerned but also the entomologist, the bacteriologist, the pathologist and the chemist.

The future of this treatment depended upon the origination and perfection of methods to continuously produce sterile blowfly larvae in numbers to supply a potential demand sufficient to treat thousands of patients. It was for service in this phase of the problem that the aid of the entomologist was sought. The Congress of the United States responded by
granting an annual appropriation for research on the entomological phases of maggot therapy, and the present investigation is a result of this action.

It was soon found that the production of sterile maggots in large numbers was only one of the problems to be considered. They had to be produced economically in order to make the treatment available to people in all financial strata. A small hospital with only one or two patients could not afford to produce its own maggots; therefore, methods for transporting sterile, viable specimens for great distances were of prime importance.

The experiments reported in this paper were made in an attempt to devise methods to produce and transport large quantities of vigorous sterile maggots economically.
II. HISTORICAL

A. Insect Therapy in General

The therapeutic use of insects dates from antiquity. Most of the reputedly medicinal species have long since disappeared from modern day materia medica, but a few still persist.

The medicinal virtues of cantharidin or "Spanish fly" have been extolled for centuries. In the past, juices extracted from blister beetles of the family Meloidae were used as an aphrodisiac and vesicatory (9). Cantharidin is officially listed in both the United States and British pharmacopoeias.

The medicinal value of bee venom has been recognized for many years. That bee keepers were more immune to arthritis than other people, was credited by early observers to the sting of bees. There has been a recent manifestation of interest in the revival of this peculiar therapy. As stated by Robinson (30), apparently the earliest attempts to use bee venom in the treatment of arthritis consisted in forcing bees to sting the victim on the affected part. At present, however, the poison sac is dissected and the venom is prepared in solution for injection and for oral administration. It is also supplied in an ointment form for topical application. In Germany there have appeared laboratories for the preparation and distribution of bee venom on a rather
large scale. A tincture made from the bodies of honey bees has also been used as an antiseptic diuretic in bladder and kidney trouble (9).

The treatment of paresis with malarial fever is a modern development in medical entomology. This form of therapy was first attempted in Europe in 1917. *Anopheles maculipennis* Meigen and *A. quadriraculatus* Say are favored vectors, while *Plasmodium vivax* Grassi and Feletti is a suitable malarial organism (30). The principle responsible for the results obtained by this form of therapy is not fully understood. The rise in body temperature, due to the malarial fever, is considered as a potent factor, and with this in mind the use of diathermy in the treatment of paresis has become widespread.

Goldstein (9) refers to the use of the cockroach *Blatta orientalis* Linn. for the treatment of dropsy by people along the Danube. In the past it was also used as wound dressings to prevent tetanus, and emulsions were administered orally.

Formic acid derived from the ant *Formica rufa* Linn. is used medicinally by some present day homeopaths (9). In South America ants have been employed by natives to suture wounds. A large and ferocious species is induced to bite through the skin of the opposing sides of the wound, after which the insect's head is severed, and the permanently locked mandibles act as efficient sutures.

Riley (26) calls attention to the use of the so-called
aleppo gall in Europe for the treatment of diarrhoeas. He assumes that any virtue possessed by the galls is due to the tannin present. A sumac gall, *Rhus glabra*, was employed by the Chippewa Indians for similar purposes (26).

The middle ages saw the use of many fantastic medicinal panaceas concocted from insects. All of these have not been forgotten. Riley (26) states that at least 30 species of insects receive recognition as of medicinal value in the United States today.

B. Maggot Therapy

The most recent contribution to insect therapy, and one which has been investigated and approved by many present day scientists, is the use of blowfly maggots in the treatment of pyogenic infections. A scientific approach was made to this subject in 1928, but earlier observations probably contributed toward establishment of the treatment.

Livingston and Prince (16) say that Ambroise Paré (23) in the sixteenth century observed unusually rapid healing of suppurating wounds in which blowflies had deposited their eggs. The following observations of D. J. Larrey (15), Napoleon's famous military surgeon, are taken from the reports of Baer (3) and Livingston and Prince (16):
During the progress of suppuration, the patients were only troubled by worms or larvae of the blue flies common in Syria. The hatching of the eggs, which these flies constantly deposit in the wounds or dressings, was assisted by the heat of the weather and by the quality of the dressings, which were of cotton, which alone could be procured in this country. The presence of these insects in the wounds, appeared to accelerate their suppuration; but they caused a disagreeable pruritis, and obliged us to dress them three or four times a day. They are produced in a few hours, and increase with such rapidity, that in the course of a night they grow to the size of the barrel of a small quill. It is necessary, at each dressing, to use lotions of a strong decoction of rue with a small portion of sage, which destroys them; but they were soon reproduced for want of proper means to prevent the approach of the flies and to destroy their eggs. Although these insects were troublesome, they expedited the healing of the wounds by shortening the work of nature, and causing the sloughs to fall off.

In spite of the importunities of these insects they have accelerated the cicatrization of the wounds by abbreviating the work of nature and in provoking the destruction of scar tissue, which they destroy.”

In his Curiosities of Medical Experience (21) J. G. Millingen says:

“During the retreat of our troops after the battle of Talavera (1809) I found the wounds of many of our men, that had not been dressed for three or four days, pullulating with maggots. This was not the case with the Spanish soldiers, who, to prevent this annoyance (which was more terrific than dangerous) had poured olive oil upon their dressings. I invariably resorted to the same practice when I subsequently had to remove the wounded in hot weather.”

Baer (3) quotes a Civil War observation by W. W. Keen as follows:

“During the Civil War maggots were very common in summer—the resulting maggots were certainly disgusting but so far as I ever observed they did no harm.”

Probably the first intentional use of maggots in surgery was by J. F. Zacharias, a surgeon in the Confederate Army.
His statement is taken from Roberts (27):

"During my service in the hospital at Danville, Virginia, I first used maggots to remove the decayed tissue in hospital gangrene and with eminent satisfaction. In a single day they would clean a wound much better than any agents we had at our command. I used them afterwards at various places. I am sure I saved many lives by their use, escaped septicemia, and had rapid recoveries."

Roberts (27) also gives the following information concerning more recent use of maggot therapy:

"About 30 years ago there was a famous surgeon in South Chicago whose name was Larkin. Whenever dressings became filled with maggots in the summer time, it was his practice to remove the dressing, clean off the wound with alcohol, brush the maggots into the wound, and re-apply the dressing. His results were good and the treatment was used not only in osteomyelitis but in chronic septic cases as well."

Baer (3) says that similar observations were made by others during the World War. It was left to Baer himself, however, to develop maggot therapy on a sound and scientific basis. As aptly stated by Buchman and Blair (4): "The gift to observe accurately is given to a few, but the gift to interpret observations properly and to apply them effectively in the solution of confronting problems is given to only an occasional student." Dr. Baer was such a student.

Baer's original observations (3):

"During the late World War an observation which I made among the wounded soldiers led me to believe that the prevention of an infection, and the curing of an infection, could be brought about by means other than chemical. At a certain battle during 1917, two soldiers with compound fractures of the femur and large flesh wounds of the abdomen and scrotum were brought into
the hospital. These men had been wounded during an engagement and in such a part of the country, hidden by brush, that when the wounded of that battle were picked up they were overlooked. For seven days they lay on the battlefield without water, without food, and exposed to the weather and all the insects which were about that region. On their arrival at the hospital I found that they had no fever and that there was no evidence of septicemia or blood poisoning. Indeed, their condition was remarkably good, and if it had not been for their starvation and thirst, we would have said they were in excellent condition. When I noticed the extent of the wounds, of the thigh particularly, I could not but marvel at the good constitutional condition of the patients. At that time the mortality of compound fractures of the femur was about seventy-five to eighty per cent.---even when the wounded had the best of medical and surgical care that the Army and Navy could provide. Later, of course, the mortality was reduced as the splinting improved in the advance area, and when finally the splinting was made to a compound fracture of this nature where the men fell, the mortality was cut down to about twenty-five per cent.

Here, however, were two men in the earlier part of our engagement in the War, when the mortality of compound fractures of the femur was high, who to all intents and purposes, were constitutionally well. This unusual fact quickly attracted my attention. I could not understand how a man who had lain on the ground for seven days with a compound fracture of the femur, without food and water, should be free of fever and of evidences of sepsis. On removing the clothing from the wounded part, much was my surprise to see the wound filled with thousands and thousands of maggots, apparently those of the blowfly. These maggots simply swarmed and filled the entire wounded area. The sight was very disgusting and measures were taken hurriedly to wash out these abominable looking creatures. Then the wounds were irrigated with normal salt solution and the most remarkable picture was presented in the character of the wound which was exposed. Instead of having a wound filled with pus, as one would have expected, due to the degeneration of devitalized tissue and to the presence of the numerous types of bacteria, these wounds were filled with the most beautiful pink granulation tissue that one could imagine. There was practically no bare bone to be seen and the internal structure of the wounded bone, as well as the surrounding parts, was entirely covered with the pink, rosy granulation tissue which filled the wound. Bacterial cultures were made and, while one found a few Staphylococci and Streptococci still remaining, they
were very few in number and not sufficient at that time to cause a pus formation. These patients went on to healing, notwithstanding the fact that we removed their friends which had been doing such noble work."

Dr. Baer "mulled" the problem over in his mind for ten years and finally decided to try treating certain of his long-standing cases of osteomyelitis with maggots. His first attempts were crude but effective. Unsterile maggots were first used, but the development of several cases of tetanus necessitated an alteration of technique. This new technique had to be adaptable to the rearing of large quantities of sterile maggots, that would be available for therapeutic use at all times. This was a new endeavor in entomology as only experimental, bacteria free maggots had been reared up to this time.

C. Animal Life Under Aseptic Conditions

La vie sans microbes est-elle possible? This is essentially the question presented to scientists by Pasteur in 1885 (24). Pasteur believed the question would be answered in the negative. The response to his query, however, brought an affirmative answer.

Baer (3) discusses the views of Nencki, who in 1886 opposed the views of Pasteur and reasoned that digestive enzymes of the pancreas, stomach, and intestines split up
food into nourishing end products without the aid of bacteria. He cited the fact that most of the end products of bacterial decomposition in the intestine, such as indol, skatol, phenol, and carbon monoxide are actually harmful to bacterial life. According to Baer (3) he predicted that chickens, dogs, rabbits, or guinea pigs might be grown aseptically. Menckil did not support his views by experimentation.

Mme O. Metchnikoff in 1901 was able to rear sterile tadpoles (18).

Delcourt and Guyénot in 1910 devised methods for rearing aseptic Drosophila on sterile media (7). According to Baer (3) these were the first workers to accomplish this feat with fly larvae.

In 1912 Cohendy (6) conducted experiments with chickens reared from sterile eggs. He divided his chickens into three groups: (a) Sterile chickens supplied with sterile air and food, (b) chickens from sterilized eggs, infected, and then fed with sterilized food and kept in a similar environment to those of group a, and (c) chickens reared normally. It was found that chickens from groups a and b were smaller and weaker than normal chickens from group c. As fowls from groups a and b showed no great difference it was concluded that the absence of micro-organisms is not in itself detrimental to the chicken.

Wollman (40) in 1922 reared aseptic larvae of Calliphora
and *Lucilia* on sterilized meat. Glazier (8) in 1924 working with several different species of flies concluded that the larval stages of flies are dependent upon certain accessory growth factors which must be ingested with the food, and that these factors are destroyed by sterilizing the food at high temperatures. They could be supplied, however by adding bacteria or yeast, as well as tissues of higher plants and animals. Possibly because these growth factors could be obtained from plant and animal tissues as well as micro-organisms, Glazier concluded that "micro-organisms and their activities are not absolutely essential to the normal growth, development and longevity of the flies investigated." This statement has subsequently been confirmed.

Michelbacher et al. (19) found that the addition of cystine to a sterile, casein base synthetic larval food, produced normal growth and development of *Lucilia sericata* Meigen larvae. Where cystine was not added to the food the pupae produced were irregular in shape.

Since the inception of maggot therapy many workers have successfully reared sterile fly larvae and some of their methods are discussed in the following pages.
III. EXPERIMENTAL

A. Materials

1. The insect

The species of fly used in this investigation was *Lucilia sericata* Meigen. Other species as *Lucilia caesar* Linn., *Phormia regina* Meigen, *Calliphora erythrocephala* Meigen, *Wohlfahrtia nuda* Weid., and even *Cochliomyia macellaria* Fabricius have been employed in maggot therapy, but *L. sericata* seems to be the best laboratory animal, and in some cases the easiest to handle in the wound. The particular strain of flies used had been inbred in the laboratory for three to five years.

a. The egg. The eggs are laid in clusters on meat. They are minute, white, elongate, and adhere to each other by a mucoid material of unknown composition. On an average 10 eggs weigh one milligram. They will hatch in eight to twenty-four hours in an oven operating at 26.5°C. In tests employing 12,580 eggs the solid content was found to be 23.2 percent of their fresh weight. This was obtained by desiccating to constant weight in a hot air oven at 100°C.

b. The larva. The larvae are spindle shaped. When first hatched they are very white, but usually become a creamier white as they grow older. According to Knipping (13), "Species of *Lucilia* in the third instar may be readily distinguished from those of *Calliphora* and *Cynomyia* by the
absence of a small rod-like structure below and between the
tips of the oral hooks, and from Phormia, Cochliomyia,
Chryaomyia, and Sarcophaga by the presence of a complete
peritreme around each posterior spiracle." The three instars
of L. sericata may be differentiated as follows:

1'. The first instar. This stage may be differenti-
tiated from the other two by its small size, about 2.0 mm.
in length, and by the small posterior spiracles, each with
two apertures but no peritreme (13). No anterior spiracles
are apparent.

2'. The second instar. In the second instar the
posterior spiracles have two apertures, but are surrounded by
an incomplete peritreme. Anterior spiracles are apparent but
smaller than with the third instar.

3'. The third instar. In this stage the larvae
are normally a creamy white and average about 14 mm. in length.
The posterior spiracles are rounded, and each contains three
slits, surrounded by a peritreme. Two anterior spiracles are
present, each divided into a number of exterior branches.
The number of branches although fairly constant was found to
vary relative to the type of substratum upon which the larvae
fed.

For a more detailed description of all three stages of
larvae the reader is referred to Knipling's paper relative to
taxonomic characters of some common Lucilia larvae (13).
c. The pupa. This is the quiescent stage in which the larva metamorphoses into the adult fly. The pupa is oval in shape and brownish in color. The color deepens as the emergence date is approached. Only by special microscopic study can it be distinguished from pupae of closely related species. Adult flies emerge when the pupae are from five to eight days old.

d. The adult. *Lucilia sericata*, the species used in these experiments, is bright green in color and averages about 8 to 10 mm. in length. If given meat soon after emergence it will oviposit in about five days. When fed on a suitable food the average longevity was found to be 43.3 days. *L. sericata* can be distinguished from *L. caesar* by the presence of three posterior acrostichal bristles instead of only two. Flies of this genus possess bare squamae and are thus distinguished from the genus *Calliphora* which possesses squamae with a dorsal pilosity (25). The genus *Phormia* when at rest holds its wings closely crossed over the abdomen making their costal margins almost parallel. This alone makes it readily distinguishable from either *Lucilia* or *Calliphora*. It is also of a larger size and darker in color. The upper side of the stem vein is ciliated, while in *Lucilia* and *Calliphora* the stem vein is bare.

As a rule the genus *Cochliomyia* should be avoided for therapeutic purposes as it contains the dangerous screw worm
fly G. americana C. and P. Laake et al (14) states that this fly may be readily distinguished from other genera of blowflies in the United States by the characteristic yellow, orange, or reddish face and the three dark stripes on the dorsal surface of the thorax.

The complete life cycle of Lucilia sericata from the egg to the death of the female averages about 58 days, although females have been kept alive for as long as 90 days.

2. The constant temperature cabinet

In order to produce flies throughout the year it is essential to have some kind of temperature-controlled rearing cabinet. Many different types are available and suitable. The one used was made of wood with glass doors and ends, and supplied with two shelves sufficient to hold 16 cages. Heat was supplied by electric light bulbs placed below and at one end of the rearing compartment. These bulbs were connected through a relay with a thermoregulator within the cabinet. Opposite and on the same level with the heating units there was a humidity chamber containing a large pan filled with water. Absorbent wicks hung into the water. A fan placed between the heater and the humidity chamber drew air via an opening in the heating chamber, over the light bulbs, and blew this warmed air into the humidity chamber. From here the air rose through holes in the floor of the cabinet to the
rearing compartment. Small openings in the top of the cabinet allowed escape. In addition, an exhaust fan was connected to cabinets where maggots were reared for removal of odors.

3. The blowfly cage

For the production of surgical maggots a special type of breeding cage has been developed by Simmons (33). The cage is the result of efforts to devise a practical and efficient type for both experimental and large-scale production.

The cage is very simply constructed. The top and bottom are circular pieces of soft wood, preferably cypress, held in place by six perpendicular iron rods. The floor of the cage is three-fourths of an inch thick. The top is one-half an inch thick and is braced on the under side with fiber-board about three-sixteenths of an inch thick. This reinforcement is necessary to prevent splitting, as there is a circular opening in the top seven inches in diameter. A water-proof cement is used between the wood and fiber-board, which prevents water from seeping between and holds the two layers together. The rods uniting bottom and top are three-sixteenths of an inch in diameter. The outside nuts on the rods are counter bored into the wood, and the top holes are closed by wooden pegs. The nuts are required on each side of the wood to hold the ends in place and insure rigidity. Should the cage become loose it can easily be made rigid again by tightening the nuts.
The rods are set in one-half an inch from the edges. Grooves one-eighth of an inch deep and about as wide are cut around the edges of both the top and bottom pieces. The purpose of these grooves is to hold a cord or rubber band used in fastening on a cloth sack. The top one is to hold the sack on, and the bottom one to prevent the flies from crawling between the cloth and the base of the cage. The lower edge of the bottom piece should be slightly bevelled to permit the cloth cover to be slipped on easily. A rounded edge also reduces wear on the cover at that point.

The entire frame with the exception of the top is covered with the cloth sack. Two different materials are used. One is a thin, white and semitransparent cloth, such as mercerized lawn, that will launder well. This is used to make the entire sack except the bottom. The other is heavy cotton suiting, a more serviceable material, and extends up around the bottom piece to the floor level of the cage. The heavy material is necessary because of the rough treatment the cage receives in being slid in and out of the incubator. A sleeve eight inches long and four inches in diameter is sewed in the sack about two and one-half inches from the bottom for the introduction and removal of food. This is far superior to a door, as the operator can slip his hand into the cage with little chance of flies escaping. In experimental work it is very important to prevent the escape of any flies. The
sleeve is closed by tying it with a string.

After a cage becomes dirty, the cloth is removed and laundered. A sufficient number of sacks should be supplied to permit laundering regularly. The cage can readily be cleaned by the use of a brush and soapy water. As this type contains a minimum number of cracks and other spaces where dirt can collect, it is very sanitary and easy to clean. When transferring flies from a dirty cage, a clean one is inverted over the other and the intervening celluloid, having previously been loosened, is removed. The cages can then be left and the flies will soon go up into the clean one. If a rapid transfer is desired a black cloth may be placed around the lower one, and the flies, being positively phototropic, will go upward rapidly into the light cage. The ease with which flies can be transferred is an important point to consider. They should be changed once or twice a week, and if many cages are used much time and labor can be saved by an efficient method.

The cage is very easily assembled for use. When clean, one has only to slip the cloth sack over it, put the bands in place and thumb-tack a piece of .015 inch thick, transparent, sheet celluloid over the opening in the top, using a sufficient number of tacks to prevent the escape of flies.

The elimination of screen wire from the cage is an important factor as wing injury seems to be directly propor-
tional to the amount of wire gauze used. A transparent top is used in order to facilitate observations and the placement and removal of food through the sleeve. Where dishes are put in blindly they are not infrequently placed on top of flies. Also, when food is being removed it is sometimes necessary to brush flies off to prevent their escape.

The size of the cage will depend upon the purpose for which production is intended and the type of incubator used. A cage ten inches in diameter and fifteen inches high is very satisfactory. This size is much smaller than that of the rectangular type sometimes used, and permits the use of a larger number to the same amount of incubator space. At the same time it is amply large to hold the necessary dishes. It is also of a convenient size to be placed in an autoclave when killing a cage of old flies in this manner, or sterilizing the cage.

The cost of construction is probably as low as for any type of substantial cage. The cost of the cloth sacks, including material and labor of sewing, is 75 to 90 cents each. The cost of constructing the frame may range from $2.00 to $3.00. Based on these prices it will be seen that a complete cage would cost only from $2.75 to $3.90. The variation in cost is largely dependent upon the labor employed.

A summary of the outstanding features of this type of fly cage follows: (1) Sanitation, and ease of cleaning;
(2) efficiency with which flies can be transferred; (3) convenience in assembling the cage; (4) no screen wire present; (5) simplicity; and (6) moderate cost.

B. Results

1. The culture of brood larvae

Brood larvae are those reared (usually under septic conditions) for maintaining the progeny.

a. The cage. A special propagation cage is essential for this purpose. A convenient one has been described by Robinson and Simmons (32), Figure I. It consists of an outer oiled paper carton of one-half gallon capacity, and an inner one of one-half pint capacity. The latter contains the food and is placed upon about one inch of washed sand. When the larvae reach maturity (5 to 8 days old) they migrate over the edge of the food container, into the sand, and pupate. The pupae are then removed to fly cages for emergence.

A certain amount of odor develops from feeding maggots. To avoid its presence in the laboratory the maggot cages should be kept in a cabinet provided with an exhaust draught.
Figure I. Rearing cage for brood larvae. One sectioned to show construction, and the other ready for use.
b. The food. Meat is used as food for the maggots and tests were conducted with various portions of raw beef to ascertain that portion most favorable for their growth and development. In selecting foods, those which were most readily obtainable for most laboratories were chosen. Tests were conducted with beefsteak, beef heart, and beef brain. The proper amount of food per unit of eggs was determined by maintaining a constant amount of meat and varying the quantity of eggs. The ratio of meat to eggs is desired rather than meat to larvae, as it would be impractical to hatch eggs on one food and later count the maggots and transfer them to another. When the largest amount of eggs per unit of meat produced normal larvae the optimum ratio was considered reached. It was found that this was approximately one milligram of eggs (10 eggs) to one gram of meat. This was approximately the same for the different foods. The ratio of meat to eggs increased as the quantity of the meat was reduced. This was due to the rapid desiccation of the smaller quantities. It was found that normal larvae could be reared on 150 grams of meat without lowering the ratio of eggs. In testing the value of the three foods used, this ratio was adopted. The maggots were reared in a cabinet maintained at a temperature of 26.5° C. and a relative humidity of 40 to 80 per cent.
Table I

Weight of Mature Larvae in Relation to Food.

<table>
<thead>
<tr>
<th>Food</th>
<th>Amount of food per test (grams)</th>
<th>Number of eggs per test</th>
<th>Total number of specimens weighed (all tests)</th>
<th>Average weight per specimen (Mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beefsteak</td>
<td>150</td>
<td>1,500</td>
<td>29,967</td>
<td>46.5</td>
</tr>
<tr>
<td>Beef heart</td>
<td>150</td>
<td>1,500</td>
<td>31,445</td>
<td>46.3</td>
</tr>
<tr>
<td>Beef brain</td>
<td>52</td>
<td>520</td>
<td>2,458</td>
<td>42.2</td>
</tr>
</tbody>
</table>

From the above table it will be seen that beefsteak, as a food, produces larvae slightly larger than either of the other two. Heart is approximately as good, but as steak is more readily accessible its use is preferred. Brain, besides being undesirable to work with, produced smaller specimens than either of the other foods. It is very important to obtain full size larvae if best results are to be obtained from the resulting adults. It has often been noticed that small undernourished larvae develop into small, poor-laying, and short-lived flies.

2. The culture of brood flies

Colonies of blowflies must be kept on hand at all times
for egg laying. Best results are obtained by keeping the flies in a cabinet maintained at the proper temperature and humidity. It has been found that a temperature of 26.5 °C. and a relative humidity of 40 to 80 per cent is satisfactory.

a. Food. Newly formed pupae placed in cages transform and emerge as flies within five to eight days, and these must be fed from this time on. The type of food is important, as it to a large extent determines the length of life of the subsequent fly, the number of eggs laid, and their viability.

In order to test the value of various foods on adult flies, eight cages containing 12 males and 12 females each were set up. The eight cages were divided into four lots of two each. Each lot was supplied with a different food. The four foods used were:

1. Water 70 parts, extracted honey 30 parts, one egg, and one-fourth cake of Fleischmann’s yeast to every 200 cc.

2. Sliced banana.

3. Granulated cane sugar.

4. Whole milk 70 parts, extracted honey 30 parts.

In addition to food, all cages were supplied with water and meat daily. Eggs were collected each day for the entire life of the flies and the number determined by weighing, allowing 10 eggs per milligram (32). Dead flies were removed daily and a record made of their sex and date of death. An even ratio of males to females was maintained in the cages at
b. Number of eggs laid. The average total number of eggs laid by a cage of 12 females fed on the honey-yeast mixture, a modification of the Baer diet (3), was higher than for flies fed on any other food, (Table II). The average total number for flies fed on sugar and banana was about equal, but considerably lower than with flies fed on the honey-yeast. Flies fed on milk-honey laid fewer eggs than did any of the other lots.

Even though the egg-laying period extended over 60 days for flies fed on the best foods, (Table II), most of the viable eggs were laid during the first 3 or 4 weeks. After this time the number dropped considerably, as did also the viability. Viability tests (32) on eggs from the various cages showed that those from flies fed on foods numbers 1 and 3 were higher in viability than the others. Almost all the eggs laid by flies fed on milk-honey failed to hatch. In consideration of the number of eggs laid, their viability, and the mortality of the flies, an optimum egg-laying period may be established for the flies fed on the various foods. With the honey-yeast mixture a longer optimum egg-laying period was obtained than with any other. Table II shows the length of the optimum egg-laying period for flies fed on the various foods. The beginning of this period was usually about the second or third day after the flies began laying.
From the results obtained it appears that the honey-
yeast mixture is better than any of the other foods tried.
Sugar, although not equaling the honey-yeast in many respects,
is very convenient and economical. It has been found a good
practice to keep a dish of it in the cages at all times, so
that should the cotton pad soaked with the honey-yeast mix-
ture dry out, the flies would always have a food supply. By
doing this one may secure good results by giving the flies
honey-yeast only about twice a week. The sugar is most con-
veniently provided in tablet form.

Banana and the milk-honey mixture are, comparatively
speaking, unsatisfactory foods when used alone.
Table II

Relation of Food to Egg-Laying Capacity and Longevity of Females

<table>
<thead>
<tr>
<th>Foods</th>
<th>Honey-yeast</th>
<th>Sugar</th>
<th>Banana</th>
<th>Milk-honey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs-laying period (days)</td>
<td>60.3</td>
<td>45.5</td>
<td>37.5</td>
<td>35.5</td>
</tr>
<tr>
<td>Total number of eggs laid (average per 12 females)</td>
<td>27,493</td>
<td>9,840</td>
<td>8,469</td>
<td>7,196</td>
</tr>
<tr>
<td>Average number of eggs laid per day per female</td>
<td>55.4</td>
<td>26.1</td>
<td>22.0</td>
<td>27.9</td>
</tr>
<tr>
<td>Average longevity of females (days)</td>
<td>43.8</td>
<td>43.0</td>
<td>36.6</td>
<td>33.4</td>
</tr>
<tr>
<td>Total days eggs were actually laid</td>
<td>33.0</td>
<td>25.0</td>
<td>24.5</td>
<td>20.0</td>
</tr>
<tr>
<td>Optimum egg laying period (days)</td>
<td>34</td>
<td>21</td>
<td>26</td>
<td>----</td>
</tr>
</tbody>
</table>

0. Effect on mortality. The different foods had a considerable bearing on the longevity of the flies (Figure II). Flies lived longer when fed the honey-yeast mixture or sugar, (Table II and Figure II). The maximum longevity of flies fed on these foods was 70 days. With milk-honey the maximum longevity was only 45 days. It will be noted that no flies died in the cages containing honey-yeast before 20 days. This is
an important feature as it is during this time that they are most valuable. Eggs laid after this period, as already stated, are not only lacking in number but also in viability. With banana the period was shorter than with honey-yeast, while flies fed on milk-honey had an average longevity of only 33.4 days, which is 10.4 days under that attained by flies fed on the honey-yeast mixture.

In summation, flies fed on the honey-yeast mixture have a longer life, a longer egg-laying period, and lay more viable eggs than those fed on any of the other foods tried.
Figure II. Mortality of female flies in relation to type of food.
3. **Culture of sterile larvae**

a. **Separation of the eggs.** The eggs are laid in clusters, and before being sterilized should be completely separated to permit the disinfectant to reach the entire surface of each, and also to prevent the loss in decanting of unseparated clumps which float to the top of the disinfectant.

A method which will separate eggs rapidly is as follows: when the eggs are removed from the meat, upon which they are laid, they are placed on a wet fine-mesh cloth on a wet cotton pad, in a covered petri dish. If the eggs are in large clumps it is best to place them on one half of the cloth, turning the other half over them. After a few minutes the eggs can be separated easily by spreading them thinly on the cloth, with a spatula. A solid, thin-blade section lifter (Figure III) is especially satisfactory for this purpose. The longer the eggs remain on the cloth, the more easily they can be separated. The use of a black cloth as a background for the white eggs permits detection of minute clumps. Eggs should be transferred to the disinfectant (Figure IV, A) by means of the spatula, after which they should be stirred gently. Thousands of eggs may be separated in a few seconds by this method. Microscopic examination showed no mechanical injury to the eggs after separation.

Some disinfectants are not suitable for use in sterili-
zation of eggs separated in this way. The mucoid covering of the egg is not removed but merely softened in the separating process, and when immersed in certain solutions the eggs agglutinate (Figure IV, B). Those solutions best adapted for use with eggs separated by this method are discussed under Disinfection.

The wet-cloth method (34) makes it unnecessary to wash the eggs, a procedure necessary after chemical separation, (6, 22, 38, 39). The elimination of this step alone saves much time and prevents the loss of numerous eggs. Chemical separation also frequently causes considerable mortality; moreover, it is not always effective. Weil et al., (38) using a chemical method of separation, state that hand-picking is necessary to remove unseparated clumps. Purely mechanical methods of separation are recommended by some workers (3, 4, 20, 37), but some of them have been found to consume too much time, and frequently have resulted in the loss of many eggs.
Figure III. Eggs being separated with a section lifter on wet black cloth.
b. Disinfection

1'. Technique. All eggs used in this series of tests were taken from the fly cages at the end of the day, stored over night in a refrigerator, and sterilized the next morning, as described by Simmons (34). This is the usual practice in maggot culture and was therefore adopted in these tests. The investigation here reported, however, has made it possible to eliminate over-night storage. The eggs two to four hours old were stored for eighteen hours at approximately 5.0° C.
Figure IV. A. Eggs in a solution of 5 per cent formalin plus 1 per cent sodium hydroxide, showing absence of agglutination. B. Eggs in pure 5 per cent formalin, showing agglutination produced.
After storage and separation the eggs were immersed in sterile test tubes containing the various disinfectants and were stirred occasionally. When the sterilization was completed a portion of the disinfectant 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.* A glass cap covered the crucible and neck of the bottle to maintain the sterility of the eggs until they were used. This apparatus is shown in Figure V. Aseptic technique was used throughout the process. About 50 cc. of sterile water was poured slowly over the eggs to remove the disinfectant. The gauze containing the eggs was then transferred to a jar of sterile food, consisting of small cubes of pig's liver or 10 per cent bacto-liver in 1 per cent agar, (29, 32). The food jars were wide-mouth specimen bottles, 77 mm. high and 44 mm. in diameter, as shown in Figure VI. Approximately 200 to 300 eggs were used in each test.

As checks upon the sterility of the maggots, both aerobic and anaerobic cultures were made of each lot. These were made after the eggs had hatched and about forty-eight hours after their disinfection, when the food had begun to liquefy. These tests were made by culturing portions of the food in

* This method was devised by Dr. G. F. White, of the U. S. Bureau of Entomology.
which the maggots were feeding. This method has proved superior to that of crushing and culturing a few of the maggots, as it is a more representative test. When taking samples for cultures the platinum loop was stabbed into all parts of the food. The cultures were incubated at 37.5° C. for seven days before the results were recorded. Robertson's cooked meat medium was used for anaerobic, and nutrient agar slants for aerobic cultures.

Tests were made of each disinfectant used, to determine its effect upon the viability of the eggs. The number of eggs used in these tests was determined by the weight method, and the number that hatched by actual count of the maggots. A portion of the eggs from each colony was allowed to hatch without being sterilized, as a control. In calculating the percentages of hatch of sterilized eggs the hatch in the control was considered as 100 per cent.
Figure V. Egg washing apparatus consisting of specimen bottle and glass cap, containing Gooch crucible and absorbent gauze.
Figure VI. Jar with sterile food for rearing surgical maggots.
2'. **Agglutination of eggs.** Since certain solutions cause agglutination of eggs, it was necessary to alter most of those used by the addition of other substances (Table III). Formalin used alone always caused excessive agglutination (Figure IV, B); and the mercuric chloride formula and phenol also caused agglutination. This is shown in Table III. Formalin to which 1 per cent sodium hydroxide was added yielded excellent results and this mixture proved to be the most satisfactory disinfectant used.

3'. **Bactericidal properties of solutions used.** In choosing a disinfectant, other factors in addition to its ability to sterilize eggs must be considered, such as egg mortality, agglutination, floating, time consumed, and age of the egg at the time of sterilization. Of the 9 disinfecting combinations used, only 1 failed to give 100 per cent sterility in routine maggot culture (Table III). From this it might be assumed that a wide range of choice is available; however, the factors mentioned limit the choice to a narrow range.
Table III

Comparative Effect of Disinfectants on Sterility and Viability of Eggs.

<table>
<thead>
<tr>
<th>Disinfectant*</th>
<th>Expos-</th>
<th>Eggs</th>
<th>Hatch</th>
<th>Ster-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sure</td>
<td>used</td>
<td>(Per</td>
<td>il-</td>
</tr>
<tr>
<td></td>
<td>(Min-</td>
<td>(Num-</td>
<td>Cent)</td>
<td>(Per</td>
</tr>
<tr>
<td></td>
<td>utes)</td>
<td>ber</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formalin 5%**</td>
<td>5</td>
<td>925</td>
<td>77.6</td>
<td>100</td>
</tr>
<tr>
<td>Formalin 5% / sodium hydroxide 1%</td>
<td>5</td>
<td>7,603</td>
<td>76.8</td>
<td>100</td>
</tr>
<tr>
<td>Formalin 5% / sodium hydroxide 1%</td>
<td>10</td>
<td>2,086</td>
<td>50.0</td>
<td>100</td>
</tr>
<tr>
<td>Formalin 10% / sodium hydroxide 1%</td>
<td>10</td>
<td>2,054</td>
<td>43.2</td>
<td>100</td>
</tr>
<tr>
<td>Formalin 5% / potassium hydroxide 1%</td>
<td>5</td>
<td>2,098</td>
<td>55.4</td>
<td>100</td>
</tr>
<tr>
<td>Formalin 5% / potassium hydroxide 1%</td>
<td>10</td>
<td>1,531</td>
<td>47.7</td>
<td>100</td>
</tr>
<tr>
<td>Formalin 5% / Labarraque's solution 2%***</td>
<td>5</td>
<td>7,833</td>
<td>40.5</td>
<td>100</td>
</tr>
<tr>
<td>Saturated solution of calcium hypo-</td>
<td>5</td>
<td>2,033</td>
<td>48.6</td>
<td>58.1</td>
</tr>
<tr>
<td>chlorite</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mercuric chloride, 1:2000, alcohol 26%, and hydro-</td>
<td>30</td>
<td>2,036</td>
<td>56.4</td>
<td>100</td>
</tr>
<tr>
<td>chloric acid 0.5%**</td>
<td>5</td>
<td>939</td>
<td>57.3</td>
<td>100</td>
</tr>
<tr>
<td>Phenol 2%**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium hydroxide 4%, for 3 hours, followed by for-</td>
<td>210</td>
<td>1,798</td>
<td>45.4</td>
<td>100</td>
</tr>
<tr>
<td>malin, 5%, for 30 minutes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Prepared fresh just before use.

** Causes agglutination of eggs following separation.

***Labarraque's solution (chlorinated soda) contains 2.6 per cent sodium hypochlorite.
Although most disinfecting solutions in general use are probably not capable of killing certain resistant spores should they be encountered, this does not necessarily impair their value as safe and practical egg disinfectants. The fact that a high sterility is regularly obtained over a long period with solutions of this type and that laboratories have successfully used such disinfectants for periods ranging from seven to eight years seems sufficient proof that resistant spores are rarely encountered when careful cultural technique is used. It should also be remembered that a sterility test, solely to detect contamination, is made of each lot of maggots before they are implanted in the wound.

To show the strength and exposure that would be necessary to destroy certain resistant bacterial forms, some of the solutions were tested (Table IV) against three spore formers, only one of which, Clostridium welchii (Migula) Holland, was pathogenic. In addition, two rather resistant non-spore-forming bacteria, Aerobacter aerogenes (Escherich) Beijerinck and Escherichia coli (Escherich) Castellani and Chalmers, were used to show the effect on vegetative forms. The technique consisted of exposing 0.1 to 0.2 cc. of a physiological saline suspension, of at least 100,000,000 organisms, to 10 cc. of disinfectant, except in the case of the anaerobes where usually 0.1 or 0.2 cc. of the broth, of a 24 hour culture, was exposed directly to 10 cc. of
disinfectant. All tests were conducted at room temperature. During exposure loops of the disinfectant were inoculated into media. The quantity of the media into which inoculations were made was sufficient to render the disinfectant inactive on bacteria.

*Bacillus mycoides* Flügge spores withstood, in numerous tests, a solution consisting of 5 per cent formalin plus 1 per cent sodium hydroxide for forty-five minutes. A combination of 10 per cent formalin and 1 per cent sodium hydroxide failed to kill them in thirty-five minutes but did kill them in fifty minutes. They remained viable after exposure for thirty-five minutes to a 5 per cent solution of phenol. Mercuro chloride, 1:2000, plus alcohol, 25 per cent, and hydrochloric acid, 0.5 per cent, killed them in thirty minutes. From eggs exposed to the 10 per cent formalin-1 per cent sodium hydroxide solution for ten minutes only a 43.2 per cent hatch was obtained. The mortality that would have been caused by fifty minutes exposure can be readily visualized. Only a 56.4 per cent hatch was obtained from eggs exposed to the mercuric chloride solution for thirty minutes; moreover, the solution caused agglutination of eggs when they were separated by the method described above. Formalin, 10 per cent, plus Labarraque's solution, 5 per cent, with a twenty minutes exposure completely destroyed *Bacillus subtilis* Conn *emend.* Przemsowski spores in only 55.6 per cent of the
tests conducted. The effectiveness of a combination of 5 per cent formalin and 1 per cent sodium hydroxide in killing vegetative forms in five minutes was demonstrated, not only in producing 100 per cent sterility of maggots in routine culture, but also in destroying Cl. welchii (vegetative form), A. aerogenes, and E. coli in every test conducted.
### Table IV

**Effect of Disinfectants on Various Bacteria.**

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Organism</th>
<th>Exposure (Minutes)</th>
<th>Sterility (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formalin 5% / sodium hydioxide 1%</td>
<td>B. mycoides spores</td>
<td>5-45</td>
<td>0</td>
</tr>
<tr>
<td>Formalin 10% / sodium hydioxide 1%</td>
<td>B. mycoides spores</td>
<td>10-35</td>
<td>0</td>
</tr>
<tr>
<td>Formalin 10% / sodium hydioxide 1%</td>
<td>B. mycoides spores</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Phenol 5%</td>
<td>B. mycoides spores</td>
<td>5-35</td>
<td>0</td>
</tr>
<tr>
<td>Mercuroic chloride, 1:2000, alcohol 26%, and hydrochloric acid 0.5%</td>
<td>B. mycoides spores</td>
<td>30-40</td>
<td>100</td>
</tr>
<tr>
<td>Formalin 5% / sodium hydioxide 1%</td>
<td>Cl. welchii</td>
<td>5-6</td>
<td>100</td>
</tr>
<tr>
<td>Formalin 5% / sodium hydioxide 1%</td>
<td>A. aerogenes</td>
<td>5-25</td>
<td>100</td>
</tr>
<tr>
<td>Formalin 5% / sodium hydioxide 1%</td>
<td>E. coli</td>
<td>5-25</td>
<td>100</td>
</tr>
<tr>
<td>Formalin 10% / Labarraque's solution 5%</td>
<td>B. subtilis spores</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Formalin 10% / Labarraque's solution 5%</td>
<td>B. subtilis spores</td>
<td>10</td>
<td>11.1</td>
</tr>
<tr>
<td>Formalin 10% / Labarraque's solution 5%</td>
<td>B. subtilis spores</td>
<td>20</td>
<td>44.4</td>
</tr>
</tbody>
</table>

*Where a range of exposure is shown, tests were made usually at five minute intervals.*
4'. Effect of sterilization upon egg viability.

The egg mortality caused by a disinfectant is one of the factors that limits its use. The solutions tested varied greatly in this respect (Table III). When eggs were immersed in a 5 per cent solution of formalin for five minutes a hatch of 77.6 per cent was obtained. The addition of 1 per cent sodium hydroxide, for preventing agglutination, produced practically no change in hatch. Increasing the time of immersion to 10 minutes caused an additional mortality, reducing the hatch to 50 per cent. The addition of either potassium hydroxide or Labarraque’s solution to formalin, although preventing agglutination, caused a high mortality.

When eggs were immersed for five minutes in a saturated solution of calcium hypochlorite a 48.6 per cent hatch was obtained; moreover, this solution was a poor disinfectant, giving only 58.1 per cent sterility. A 20.4 per cent greater mortality was obtained with the mercuric chloride solution than with formalin, and agglutination frequently occurred. Phenol caused agglutination and high mortality, both factors restricting its use. With the formula and technique used by White (39), namely, immersion in a 4 per cent solution of sodium hydroxide for three hours, followed by immersion in a 5 per cent solution of formalin for thirty minutes, 45.4 per cent of the eggs hatched. The time involved and the mechanical loss of eggs are other factors limiting the use-
fulness of this procedure.

The high hatch (76.8 per cent) after the eggs were immersed in a solution consisting of 5 per cent formalin plus 1 per cent sodium hydroxide for five minutes and the fact that this combination prevents agglutination and gives good bacteriological results in routine maggot culture are points in its favor.

5'. Mortality of eggs in relation to age at the time of sterilization. An investigation was made to determine the effect of sterilization on the viability of eggs of various ages. To obtain eggs of known age, meat was placed in the fly cages and allowed to remain two hours, after which a known number of the eggs laid on it were sterilized. The remainder were kept in the fly cabinet, but not in the cages, and a known portion of them were sterilized at two-hour intervals until hatching began. The number of eggs that hatched was determined by counting the maggots. Two complete tests were made with each of two disinfectants.

Sterilization of freshly laid eggs is very desirable but is possible only with certain disinfectants. The hatching results obtained with fresh eggs (up to two hours of age) after sterilization for five minutes in a combination of 5 per cent formalin plus 1 per cent sodium hydroxide were as good as when eggs were held over night in cold storage before being sterilized, as is the general practice. A better hatch
was obtained with fresh eggs than with those held from four to six hours in the fly cabinet before sterilization. The mortality of eggs disinfected just before hatching was approximately 90 per cent.

In contrast to the results obtained with the 5 per cent formalin-1 per cent sodium hydroxide solution, it was found that mercuric chloride, 1:2000, plus alcohol, 25 per cent, and hydrochloric acid, 0.5 per cent, when used for thirty minutes on eggs up to two hours old, destroyed 98 per cent of them. This solution gave best results on eggs from four to six hours old. Bushman and Blair (4), using practically this formula, found that the hatch from eggs sterilized immediately after being laid was not so good as from those in which sterilization had been delayed for several hours. Hobson (11), using 0.1 per cent mercuric chloride for fifteen minutes, stated that eggs may be sterilized the same day they are laid if first incubated at 37° C. for six hours. White (39), recommending immersion in 4 per cent sodium hydroxide for three hours followed by immersion in 5 per cent formalin for thirty minutes, stated that when eggs are sterilized too soon after being laid or when too near hatching the mortality is relatively high. Eggs up to two hours old were subjected to this treatment and the results confirmed his statement that at this age practically all the eggs were killed.

The results here presented show that when 5 per cent
formalin plus 1 per cent sodium hydroxide is used, freshly laid eggs may be sterilized with little effect upon their viability. Apparently, the more rigorous the technique, the greater the mortality with fresh eggs. It is often convenient to sterilize eggs soon after they are laid, as is shown later in this dissertation.

61. Discussion of disinfection. In the greater number of cases in which maggot therapy is employed only sterile maggots are used. A certain difference of opinion exists, however, as to the bacteriological precautions necessary in maggot culture. On the one hand McKeever (17) states that he has found sterilization of maggots to be unnecessary, as he has safely used non-sterile maggots in wound treatment. He maintains that a prophylactic injection of tetanus antitoxin before implantation of the maggots is sufficient precaution. He does not consider *G. welchii* of any importance, as Baer had previously shown the organism incapable of development in association with maggots in experimental animal wounds. He also refers to the work of Livingston and Prince (16) who, he says: "Used unsterile maggots extensively----and there have been no unfavorable results in a large series of cases recently reported by him." Grantham-Hill (10) has used non-sterile maggots in the treatment of infected wounds of natives in Sudan, Africa, and states that no ill effects have been observed. The tendency to use non-sterile maggots
is partly because of the cost of the sterilizing process and partly because of the simplicity and convenience of non-sterile maggot culture. On the other hand White (39) feels that bacteriological precautions should be made still more rigid than at present. He maintains that the purpose of disinfection is to destroy spores as well as vegetative forms of all bacteria that might conceivably be encountered. In accordance with this view he has adopted a rigorous sterilizing technique. Unfortunately, however, such a procedure prolongs the disinfecting process and destroys a considerable number of eggs, both of which factors increase the cost of sterilization.

Sterile maggots are regularly produced in vast numbers, although the egg disinfectants as now used are incapable of destroying all resistant spores. The reason for this sterilility is that certain bacteria are not usually encountered in the cultural technique. Such maggots have now been in surgical use for seven to eight years, and no case of infection introduced through this method has been found. To show the difficulty of attempting to kill all bacteria without also destroying the egg, the following data are given. Tetanus is killed in the spore stage by a 5 per cent solution of carbolic acid only after exposure for twelve to fifteen hours. To kill it in a 1 per cent solution of mercuric chloride requires from two to three hours. Anthrax spores will remain
and does not increase the burden of severity for the patient. Consequently, the diagnosis of gastric ulcer seems unnecessary, as the cause is a more frequent manifes-
tation before use to a standard procedure in general use of an.

sterility tests) both aerobic and anaerobic (made of all

procedure with the live-minute immersion in 5 per cent formalin
and then found that they can be produced repeatedly and economi-

The writer emphasizes the use of only sterile megeotes,

function of the affected part.

excellent results were obtained with an early return of

dangerous results need be feared, both bear and well round

evidence that even if "sterility tests" should reach the wound, no

super-free medium, rich in protein, such as albumin, says

that most strains sporulate only when grown on an extractive,

in the absence of fermentable carbohydrates and nitrogen says

Jordan states that spores are formed promptly and only

treatment is readied seen. In regard to "sterility tests," the E. coli

hour (or 12 hours) produces a typical loxosceles, is used for forty days, but mercuric ortho.

able after exposure to 5 per cent solution of merbado
7. **Suggested procedure in “one-day” disinfection of eggs.** By the method to be described, eggs may be laid, collected, sterilized, and placed in the incubator within one day. This results in shortening the developmental period, from egg to surgical maggot, about eighteen hours, under the usual method of collecting eggs one day, holding them in cold storage over night, and sterilizing them the next day. In tests conducted it has been found that the meat upon which the eggs are laid does not need to be exposed to the females during the whole of the day in order that a sufficient number of eggs may be obtained. Only young flies (up to three weeks of age) should be used as laying stock, the eggs of older flies being fewer in number and low in viability. The meat should be withheld every alternate day. If only a small surface is available several hours must elapse before all the flies have access to it. Larger pieces of meat than ordinary should be used so that all flies will have access to its surface for egg laying. Under these circumstances flies have laid as many eggs during the first two or three hours as colonies formerly laid during the whole day. Meat left longer than this time has apparently only a nutritional value.

A rapid method of separating the egg masses is essential under the one-day system and the wet-cloth method described herein has been found very satisfactory.

There is no time under this schedule to allow the
eggs to age two or three hours before sterilization, a procedure necessary with some sterilizing solutions. With the disinfectant recommended, namely 5 per cent formalin plus 1 per cent sodium hydroxide, the eggs may be sterilized without aging.

Under the one-day method as outlined, the meat may be placed in the fly cages about 9 A. M. and removed about noon. At this time the eggs should be placed in the separating dish (Figure III). After a few minutes the eggs may be separated and disinfected by immersion for five minutes in a freshly prepared solution of 5 per cent formalin plus 1 per cent sodium hydroxide. Following this the eggs are transferred to the sterile food jars and placed in the incubator for hatching.

4. Retardation and transportation of sterile maggots

a. Retardation by low temperature. Efficient retardation of development in the culture of surgical maggots permits the technician to maintain a constant supply with minimum effort. It is also one of the chief means by which cost of production can be lowered. As previously stated (35) retardation is essential during the period of incubation in the tests of sterility, and it would be both economical and convenient if development could be restrained beyond this period. As subjection to low temperature has been the chief
means of retardation, an investigation was made to determine its effect on maggots in storage.

A common practice of surgeons requiring a small or occasional supply of maggots is to purchase them from certain medical supply houses, and frequently shipments have to be made over long distances. Normally maggots develop rapidly, and if allowed to grow in transit they become too large for use in the wound. Their development has to be retarded in some way, therefore, during shipment. The usual method of preparing maggots for shipment is to pack the container next to ice in an insulated package. The package, however, is heavy and bulky and is expensive to prepare and mail. The greatest objection raised by surgeons using maggots for treatment has been the expense, many of their patients being dependent on charity. With a view to the reduction of expense, therefore, an investigation was also made of the efficiency of retardation by low temperature in transit.

Maggots used in this work were reared under the usual aseptic technique.

1. Proportion of maggots that feed at various temperatures. Both in storage and in transit, restraint of growth is of primary importance. Tests were therefore conducted to determine the proportion of maggots that feed under various temperatures which might be chosen for the
production of retardation.

Maggots of surgical size (from 4 to 6 mm. long) were removed from their food and subjected to the temperatures shown in Table V. They were then placed on colored food of the same temperature and returned to the refrigerator. Thus no feeding occurred except under controlled conditions. After approximately twenty-four hours all living specimens were examined microscopically for the presence of food in the alimentary tract.

The colored food was prepared by making a solution of normal beef blood serum and adding enough safranine to produce a deep red color. After being mixed, the food was coagulated by heating and placed in the food bottles for autoclaving. Even slight feeding was detected in the intestinal tract, and tests proved that maggots fed readily on this food.

Observations indicated that there was feeding as long as activity persisted. Little feeding occurred at $4^\circ$ C.; while at $8^\circ$ to $9^\circ$ C. 53.2 per cent of the maggots fed (Table V). When the temperature was increased to $10^\circ$ or $11^\circ$ C. the proportion was increased to 80.6 per cent. Almost all the maggots (98.5 per cent) fed when a temperature of $20^\circ$ C. was reached. The quantity of food consumed increased, of course, with the temperature.
Table V

Relation of Temperature to the Number of Maggots that Feed.

<table>
<thead>
<tr>
<th>Temperature, Degrees, C.</th>
<th>Number of Maggots used</th>
<th>Maggots that Fed</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-6</td>
<td>381</td>
<td>17</td>
<td>4.4</td>
</tr>
<tr>
<td>8-9</td>
<td>62</td>
<td>33</td>
<td>53.2</td>
</tr>
<tr>
<td>10-11</td>
<td>262</td>
<td>216</td>
<td>81.6</td>
</tr>
<tr>
<td>20-21</td>
<td>198</td>
<td>195</td>
<td>98.5</td>
</tr>
<tr>
<td>27-29</td>
<td>109</td>
<td>107</td>
<td>98.2</td>
</tr>
</tbody>
</table>

2'. Growth of maggots at various temperatures.

In tests already mentioned, 53.2 per cent of the maggots fed at a temperature of 8° to 9° C. The amount of feeding, however, was negligible. The actual amount of growth, therefore, and not merely the indication of feeding, is the criterion to be used in establishing a temperature suitable for retardation. Tests were conducted to determine this temperature.

Maggots were taken from their food (composed of dehydrated liver, agar, yeast and water) and washed with distilled water for one minute to remove particles of food. The surface water was removed by allowing the maggots to crawl over filter-paper for four minutes. They were then counted, weighed and placed in the refrigerator until chilled to storage
temperature. After this they were placed on fresh food of
the same temperature, returned to the refrigerator and allowed
to feed for seventy-two hours. This period was chosen because
it is that for which the cultures used in the sterility tests
are usually incubated before the maggots are released and
because it represents about the average time required for
maggots in transit. Growth during storage was determined by
removing the specimens and washing and weighing them as
before. Four tests with 100 maggots each were made for each
range of temperature indicated in Table VI.

At a temperature of 10° to 11° C. the increase in growth,
namely, 23.4 per cent of the prestorage weight, can be toler-
ated without materially shortening the period of feeding of
the maggots after implantation in wounds. A temperature of
13° to 14° C. caused an increase of 90.7 per cent in weight.
A higher temperature is, therefore, unsatisfactory, as maggots
showing an increase in weight beyond 23.4 per cent would be
excessively large after storage and would have only a short
period of feeding in the wound.

On the basis of these tests, a maximum effective temper-
ature was fixed at 12° C. as the highest that can be toler-
atured in the retardation of maggots.
Table VI

Relation of Temperature to Growth of Surgical Maggots During Seventy-two Hours.

<table>
<thead>
<tr>
<th>Temperature, Degrees, C.</th>
<th>Mean Prestorage Weight per Specimen, Mg.</th>
<th>Mean Post-storage Weight per Specimen, Mg.</th>
<th>Mean Gain in Weight per specimen during Storage, Mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-11</td>
<td>4.7</td>
<td>5.8</td>
<td>1.1</td>
</tr>
<tr>
<td>13-14</td>
<td>4.3</td>
<td>8.2</td>
<td>3.9</td>
</tr>
<tr>
<td>15-16</td>
<td>4.1</td>
<td>16.9</td>
<td>12.8</td>
</tr>
<tr>
<td>20-21</td>
<td>6.5</td>
<td>22.4</td>
<td>15.9</td>
</tr>
</tbody>
</table>

3'. Effect of retardation by low temperature on viability of maggots. The mortality of maggots during storage is of much importance. Although low temperature is used extensively as an agent to induce retardation it also causes a high mortality. If a high death rate during retardation could be avoided, maggots could be held in an arrested state for several days. This would considerably reduce the expense of production and, by permitting long distance shipments, would make surgical maggots more available to remote districts. Tests were conducted to determine whether the death rate is so great as to make the continued use of this method of retardation advisable.

The maggots used in these tests were reared on the
nutrient food already mentioned, under the usual aseptic precautions. When they reached surgical size they were stored in the same kind of food at a temperature of 5° to 6° C. for periods ranging from one to six days.

The period of mortality may be divided into two phases: (1) the time from the hatching of the eggs until the maggots are implanted in the wound and (2) the period after implantation. Maggots that survive cold storage are not necessarily able to resume feeding when placed in the wound, and obviously they would be of no clinical value if unable to feed. As a test of the tolerance of maggots to cold storage, it was therefore required that they should be able to feed and grow when placed on necrotic tissue.

Cold storage of surgical maggots caused an extreme mortality as shown in Table VII. After two days storage, which is the usual period of retardation during the sterility tests, the mortality was 66.6 per cent. The fact that two-thirds of the maggots were lost during this period of storage is significant, and as the death rate increased rapidly each day, the futility of prolonged storage is evident. When maggots were retarded for six days the mortality was almost 100 per cent, while maggots reared under similar conditions but not subjected to cold had a mortality of only about 19 per cent.
Table VII

Mortality of Surgical Maggots Resulting from Cold Storage at 5° to 6° C.

<table>
<thead>
<tr>
<th>Time in Storage Days</th>
<th>Number of Experiments Conducted</th>
<th>Number of Maggots Used</th>
<th>Number that Survived Storage</th>
<th>Number Able To Resume Feeding</th>
<th>Percentage of Mortality*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>3,006</td>
<td>2,655</td>
<td>1,104</td>
<td>63.3</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>2,764</td>
<td>1,650</td>
<td>922</td>
<td>66.6</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>2,823</td>
<td>1,112</td>
<td>604</td>
<td>73.6</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>2,183</td>
<td>781</td>
<td>397</td>
<td>81.8</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>985</td>
<td>391</td>
<td>119</td>
<td>87.9</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>448</td>
<td>56</td>
<td>7</td>
<td>98.4</td>
</tr>
</tbody>
</table>

* The number includes those that were dead when removed from storage and those not able to resume feeding.
The enormous death rate of surgical maggots caused by cold storage often escapes detection, and the fact that about one half of those which do survive storage are unable to resume feeding is still more likely to be overlooked. Another method of retardation is possible which eliminates the use of low temperature. This method, to be discussed subsequently, considerably reduces the expense and labor involved in the culture of maggots and, by lowering the mortality of the maggots, increases their therapeutic value.

4'. Retardation of maggots in transit by low temperature. When maggots are purchased, shipment by mail is usually necessary and the essential retardation during transit has been attempted chiefly by packing in iced containers.

To test the efficiency of this method, determinations were made of the temperatures within the packages, and these temperatures were compared with the maximum effective temperature of 18° C. Two standard types of packages were prepared for shipment in the usual way. One type was a cylindrical carton having metal ends and insulated with corrugated paper and cellulose. Some of these cartons were placed on end and others on their sides, as in transit. The second type was a rectangular cardboard box filled with ground cork for insulation. Ice was supplied in the usual way, by freezing water in tin cans of a capacity of 245 cc. Each bottle of maggots, with the usual small quantity of nutrient food, was packed
next to the can of ice.

The temperature of each bottle after packing was determined with a four point multiple thermocouple placed in the bottle in the following positions: Point 1, in contact with the base of the cotton plug; point 2, suspended in mid-air between the base of the bottle and the plug; point 3, fastened to the inner wall of the bottle on the same level as point 2; and point 4, inserted into the food.

Readings were taken at one-half hour or one hour intervals, and tests were conducted under the shipping conditions of both summer and winter.

a'. Tests under summer conditions. The tests were made in July with atmospheric temperatures of 30° to 33° C. (Figure VII). The only temperature in the cylindrical iced package low enough to produce successful retardation was recorded by point 4 (Figure VII), which was inserted in the food next to the ice. This temperature was 12.1° C., but it lasted only about one hour. The temperatures in the upright and prone packages were similar, and their averages were taken. About the cotton plugs it was always warmest, the mean minimum temperature being 24° C. In sixteen hours the mean minimum temperature was 27.5° C., at which temperature maggots are always active and feeding. Thus it is evident that under summer conditions practically no retardation of the maggots is accomplished by this method.
The temperatures in the rectangular package were more uniform (Figure VIII) but higher. The food was always warmest and the wall of the jar coolest, but the difference in temperature was only about 3°C. The mean minimum temperature was 18.5°C, which was not sufficiently low to produce dormancy of the maggots.

These tests were purposely conducted under the most unfavorable atmospheric conditions of summer, so that the adequacy of retardation by low temperature for the conditions of this season and of tropical countries might be determined.

b'. Tests under winter conditions. Winter temperature was obtained by placing the package in a mechanical refrigerator maintained at 20°C. This temperature was chosen as that usually encountered by maggots in mail-coaches and post-offices. The insulation of the bottle prevents chilling when the package is exposed for brief periods outside.

Under these conditions the temperatures in the bottles were both lowered and prolonged. In the cylindric containers the temperatures bore a parallel relationship to those under summer conditions. The lowest temperature, 4.5°C, was reached in the food (Figure IX). By the first hour this had risen to 7.5°C, but no appreciable increase occurred before the end of fourteen hours. A uniform temperature of 17.5°C was recorded throughout the bottle in twenty-three hours, and it was approximately that of the surrounding atmosphere in
thirty hours. All portions of the bottle were sufficiently cooled to produce a successful degree of dormancy, but only for fifteen hours. The duration of the retardation-inducing temperature in the warmest portion of the bottle was only about eight hours, and as is shown later in this paper, it is naturally this portion in which the maggots tend to cluster.

In the rectangular package the temperatures of the bottle were lower and rose more slowly than under summer conditions. The variation in temperature between the different parts of the bottle (Figure X) was only 2.5° C., and all temperatures had the same relationship to each other as those under summer conditions. In this type of package the temperatures also fell and rose more slowly than in the cylindrical container. The lowest temperature was recorded on the side adjacent to the ice. There an effective retardation-inducing temperature was maintained for eighteen hours, while in the food the time of retardation was reduced to fourteen hours. A successful degree of retardation of the maggots was obtained in all portions of the bottle, but the low temperature did not persist long enough to permit successful long distance shipping.
Figure VII. Temperatures in cylindric iced shipping package under summer conditions.
Figure VIII. Temperatures in rectangular iced shipping package under summer conditions.
Figure IX. Temperatures in cylindrical iced shipping package under winter conditions.
Figure X. Temperatures in rectangular iced shipping package under winter conditions.
5'. **Effect of temperature gradient on migration of maggots.** As the temperatures in a bottle of maggots varied when placed next to ice, it was assumed that the specimens would migrate to the warmest part. The lower temperatures would thus be ineffective. Tests were therefore conducted to determine this point.

Bottles containing food and maggots were placed on cans of ice, and the activity of the maggots was observed as the temperature fell.

In each case the maggots began to wander from the food when it was chilled to 18° C. They fed readily, however, at this temperature if no higher one was available. Further chilling to 14° C. caused most of them to leave the food and cluster in the warmer parts near the plug, and they returned to the food only when it had become warm. In Figures VII, VIII, IX, and X it is shown that this migration considerably reduced the effectiveness of the retardation-inducing temperature during shipment. On the basis of this migration, retardation lasted only about fourteen hours under the most favorable conditions (Figure X).

Many of the foods used, unfortunately, tend to run to the warmer parts of the bottle, thus becoming available to the maggots and promoting rapid growth during most of the period in transit. The tendency, therefore, is toward either overgrowth or excessive mortality, either of which prevents satis-
factory utilization of this method.

61. Undercooling and freezing points of maggots.
Tests to determine the temperatures at which the maggots would freeze were made with a thermocouple by the contact method, as devised by Robinson (28). The specimens used were of the typical surgical size (from 4 to 6 mm. in length). A summary of the data is given in Table VIII.

As freezing cannot occur until the undercooling point is reached, it is seen that freezing is impossible under ordinary conditions of cold storage.

Table VIII

Undercooling and Freezing Points of Surgical Maggots.

<table>
<thead>
<tr>
<th></th>
<th>Undercooling Points, Degrees C.</th>
<th>Freezing Points, Degrees C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum temperature</td>
<td>-14.6</td>
<td>-7.2</td>
</tr>
<tr>
<td>Minimum temperature</td>
<td>-10.0</td>
<td>-4.1</td>
</tr>
<tr>
<td>Average temperature</td>
<td>-11.4</td>
<td>-5.6</td>
</tr>
</tbody>
</table>

71. Comments on retardation by low temperature.
The customary method of retardation of surgical maggots by low temperature is inefficient. After two days of storage at 5°
to 6°C. only one third of the maggots were able to resume feeding under conditions similar to those in the wound; a period of retardation of this length is necessary during the sterility tests. After an additional storage of four days, as would be required during shipment or when surplus maggots were held on hand, less than 2 per cent fed.

The use of iced containers for maggots in transit is not only expensive but inefficient. In summer no significant degree of retardation is obtained by ice alone. In winter a certain degree of retardation is possible. This may last as long as eight to eighteen hours but this time is not sufficient to permit successful shipping for long distances.

At temperatures between 15°C and 21°C. maggots gained about 75 per cent in weight during seventy-two hours. This range of temperature is always available to them under shipping conditions in summer and during most of the time under conditions in winter.

Since the completion of this investigation, one medical supply house has used a larger ice can (with a capacity of 445 cc.), which somewhat prolongs the duration of the retardation-inducing temperature. However, if the period were to be increased three times, there would be only about two days of retardation under the most favorable conditions, and as already shown, only one third of the maggots would be able to resume feeding. The increased quantity of ice also adds con-
siderably to the weight of the package and the cost of mailing.

In the cases in which packing with ice has been employed with apparent success, the results have been largely due to the fact that the maggots' food was sufficiently low in nutrient value to aid greatly in the retardation. This conclusion was arrived at after having received and examined specimens shipped from medical supply houses.

In view of the inadequacy of retardation by low temperature a more satisfactory method is desirable. In the following section a method of effecting retardation by nutritional means alone is described. With this new method the disadvantages are less than with cold storage and the period of successful retardation is considerably longer.

a. Retardation by nutritional insufficiency. In view of the inadequacy of low temperature in the retardation of surgical maggots experiments were conducted to devise a form of nutritional retardation. The idea in view was to find a food sufficiently low in nutritional value to permit only slow growth and development, yet capable of maintaining maggot viability. If this could be accomplished the use of low temperature would not be necessary.

1'. The retardation-inducing food. There are various foods by which the growth of maggots can be retarded, but most of these cause considerable mortality or lack some of
the essentials to be enumerated.

As previously stated by the writer (36), a good food for this purpose should conform to the following standards: (1) The food must permit only a slow rate of growth; (2) it must at the same time retard development in conformity with the slow rate of growth; (3) the mortality during retardation should be slight; (4) the food should be nutritionally homogeneous throughout to allow uniform growth of all the maggots; (5) it should be of the proper consistency to permit free activity of the maggots; (6) it should permit the easy removal of the maggots; (7) it should resist drying; (8) the food materials should be accessible to the laboratory; (9) the food should be simple, cheap and easy to prepare.

A wide range of foods was tested. The one which proved to be the best and simplest is prepared as follows: Evaporated milk (fresh), 1 part*: distilled water, 7 parts; agar, 1.5 per cent.

The milk and water are mixed; the agar is then added, and the mixture is cooked in a double boiler for about twenty-five minutes. It should be poured into the food bottles while hot. Ordinarily, from 12 to 15 cc. are used in each bottle.

* The proportion used was based on the usual commercial concentration, which is about twice that of whole milk.
This proportion of milk to water is sufficiently nutritious to sustain the larvae without allowing material growth and development. Some latitude in concentration is tolerated, however, any ratio of 5 to 7 parts of water to 1 part of milk being suitable. Solutions of less than 1 part of milk to 7 parts of water are not satisfactory, as the milk tends to settle out during autoclaving so that it is overlaid with clear agar. This condition is not desirable, as newly hatched larvae should have immediate access to the milk.

A 1.5 per cent solution of agar gives a jelly-like consistency which is sufficiently soft to allow free activity of the maggots and yet is firm enough to prevent running. The agar acts as a hydrophilic colloid and thus prevents undue desiccation of the food. This action is important, as lack of moisture is detrimental to the maggots. The specimens are easily removed from the food by pouring sterile water into the bottle and stirring with an applicator, after which the maggots are strained out. If cold water is used the maggots are subdued so that they will not escape from the wound before the cage is applied.

2'. Growth and development of surgical maggots on retardation-inducing food. The rate of development as well as the rate of growth must be reduced by the retarding agent. Although the two functions usually occur simultaneously, they can be separated. If growth alone were arrested and
development continued, the maggots would be useless surgically after about five days, even though they were no larger than normal surgical maggots, for they would not feed in the wound. On the other hand, if growth were well advanced before implantation, one of the outstanding activities of the maggots, namely, the removal of necrotic tissue in feeding (31) would be correspondingly reduced.

In the evaluation of the food it was therefore necessary to determine the rate of growth and development of maggots during various periods of retardation. This was done by making measurements of size and determinations of instar every twenty-four hours until the period of successful retardation had passed.

It was found that limitation of both growth and development could be effected (Table IX) without allowing the maggots to grow beyond the recommended surgical size of 4 to 6 mm. in length. On the eighth day the average length of the maggots was only 6.3 mm. Then followed a period of slight shrinkage. While a small percentage of the maggots developed into the third instar after one week, the majority remained in the second instar throughout the period of retardation. As feeding takes place chiefly in the third instar, it is evident that the maggots would have four or five days for feeding after implantation.

These tests were conducted with the usual number (from
500 to 700) of sterile maggots in each bottle. When an extremely small number was used, however, growth was slightly accelerated.

Table IX

Growth and Development of Maggots on Retardation-Inducing Food.

<table>
<thead>
<tr>
<th>Age of Maggots from Sterilization of Eggs, Days</th>
<th>Length (mm)</th>
<th>Percentage of Maggots in Each Instar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First</td>
</tr>
<tr>
<td>1</td>
<td>2.0</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>2.8</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>4.3</td>
<td>--</td>
</tr>
<tr>
<td>4</td>
<td>5.3</td>
<td>--</td>
</tr>
<tr>
<td>5</td>
<td>5.6</td>
<td>--</td>
</tr>
<tr>
<td>6</td>
<td>6.0</td>
<td>--</td>
</tr>
<tr>
<td>7</td>
<td>6.0</td>
<td>--</td>
</tr>
<tr>
<td>8</td>
<td>6.3</td>
<td>--</td>
</tr>
<tr>
<td>9</td>
<td>6.2</td>
<td>--</td>
</tr>
<tr>
<td>10</td>
<td>6.0</td>
<td>--</td>
</tr>
</tbody>
</table>

3'. Mortality during retardation. As previously mentioned, two periods of mortality must be considered as a result of retardation: (1) the time from the hatching of the eggs to the implantation in the wound, during which the

* On the ninth day slightly fewer maggots appeared in the third instar than on the eighth day. This may be attributed to the incidental results of the random sampling.
maggots have the retardation-inducing food and (2) the interval after transference to the wound. The second period will be discussed in the following section.

In determining the mortality during the first period, a series of bottles of sterile specimens was prepared, each bottle containing equal numbers of eggs by weight. On the third day of retardation the maggots from one third of these bottles were counted; the others were counted on the sixth and ninth days, respectively. The average mortality for each series is shown in a succeeding paragraph, but a certain degree of fluctuation occurred within the series.

The total number of live specimens from the three day series was 861; no dead maggots were seen. With retardation by low temperature for three days, however, there was a mortality of 60 per cent. On the sixth day the total number of live maggots was 762, showing a mortality of 11.5 per cent due to nutritional retardation as compared with that of 87 per cent due to retardation by low temperatures. The total number of maggots in the nine day series was 727, which is a decrease of 16.6 per cent from that in the three day series. With retardation by low temperature all the maggots were killed in nine days.

4'. Effect of nutritional retardation on feeding in the wound. As retardation is an unnatural process, unfavorable effects which might manifest themselves after implan-
tation of the maggots in the wound had to be considered. If maggots were to die prematurely in the wound or fail to feed vigorously, their therapeutic value would, of course, be correspondingly reduced. It was therefore essential to determine (1) the proportion of maggots that resume feeding when transferred to necrotic tissue, (2) the amount of tissue consumed, and (3) the length of time the maggots feed. These points are discussed in the following subsections.

The laboratory tests described were supplemented by clinical observations made on patients at the George Washington University Hospital, the Gallinger Municipal Hospital, and the Emergency Hospital in Washington, D.C., to which nutritionally retarded maggots were supplied for the purpose. The results were confirmed in numerous instances by surgeons in various parts of the United States to whom nutritionally retarded maggots were supplied.

Twenty-seven bottles of sterile maggots were prepared, and as a check the tests were conducted in two series. After the maggots were 5 days old, a known number of several hundred from each of a number of bottles was transferred to a definite quantity of decomposing beef. This procedure was repeated with maggots 5, 6, 7, 8, and 9 days old. The containers of meat were placed on sand, which was examined each day for maggots. The meat was also examined for dead maggots. Any maggots left in the meat after five days of feeding were removed and counted.
By this method the proportion able to resume feeding and the length of the period of feeding were determined.

In each series additional containers of meat, without maggots, were prepared as controls to determine the natural loss of weight by desiccation. The average weight of meat per container was 144.2 grams, and the maximum variation between individual containers did not exceed 1.4 grams. This was true of the containers both with and without maggots. The loss in weight averaged about 37 per cent, and with this factor known, the quantity of tissue consumed by the larvae could be determined. It is probable that the amount of desiccation from the two groups of food differed slightly, owing to the activity of the maggots in the one group. This variation, however, apparently had little practical bearing on the results obtained.

a'. Proportion of maggots able to resume feeding.

Practically no injury to the larvae was evident after three days of nutritional retardation. This is shown by the fact that 79.6 per cent of the maggots that hatched developed to maturity when placed on necrotic tissue (Table X). This proportion is as great as the number which ordinarily mature when reared on nutrient cultural food. When retarded by a low temperature of 5° to 6° C. for three days, however, only 21.4 per cent were able to resume feeding.

There was little additional mortality after five days
of retardation, as 76.6 per cent of the maggots developed to maturity when placed on necrotic tissue. This number is in striking contrast to that of maggots retarded for the same length of time by low temperature, of which only 12.1 per cent were able to resume feeding. After six days this number was reduced to 1.5 per cent, while with nutritional retardation 70 per cent of the maggots were able to continue feeding.

After seven days of retardation a considerable drop occurred in the percentage of maggots which were able to feed. On the basis of the number that hatched, 51.8 per cent were able to feed and develop to maturity, while after eight days the number was reduced to 45.3 per cent. This proportion is twice as great as the number which were able to feed when retarded for only three days by low temperature. It is not possible to keep maggots under low temperature in a condition in which they will feed after seven days.
Table X

Effects of Nutritional Retardation and Retardation by Low Temperature on the Number of Maggots Able to Resume Feeding and on the Amount of Tissue Removed.*

<table>
<thead>
<tr>
<th>Retardation Period, Days</th>
<th>Percentage of Maggots Able to Feed</th>
<th>Necrotic Tissue Consumed per Hundred Maggots, Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nutritional Retardation</td>
<td>Retardation by low Temperature</td>
</tr>
<tr>
<td>3</td>
<td>79.6</td>
<td>21.4</td>
</tr>
<tr>
<td>5</td>
<td>76.6</td>
<td>12.1</td>
</tr>
<tr>
<td>6</td>
<td>70.0</td>
<td>1.5</td>
</tr>
<tr>
<td>7</td>
<td>51.8</td>
<td>0.0</td>
</tr>
<tr>
<td>8</td>
<td>45.3</td>
<td>0.0</td>
</tr>
<tr>
<td>9</td>
<td>28.1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* The results are based on a definite number of maggots at hatching.
At the end of nine days 28.1 per cent developed to maturity when given the proper food. The mortality incurred after this time is often compensated for by the convenience of holding over sterile maggots for future use.

The usual mortality in a bottle of maggots is extremely difficult to detect and therefore frequently overlooked. Because dead maggots are sometimes moved about or obscured from view by the activity of living ones, the assumption is often made that they are all alive. On making actual counts, however, it is frequently found that a large number are dead. This is especially true if the maggots have just died and discoloration has not yet taken place. After retardation by low temperature or after prolonged nutritional retardation many maggots die and disintegrate. The diminution in the number of live maggots in this manner is not readily noticeable, as even 100 or 200 live maggots in a bottle appear to be a great number.

b'. Amount of necrotic tissue removed. In the final analysis of the effect of retardation on the therapeutie value of maggots, the quantity of necrotic tissue which they can remove from the wound is of primary importance. A unit of 100 newly hatched maggots removed 6.4 grams of beef tissue after three days of nutritional retardation (Table X). In reality, 100 living maggots would remove more than this, but as there is a slight mortality through
retardation, less than this number were available. The usual osteomyelitic wound may require an implantation of 500 maggots, and in this case 32 grams of necrotic tissue would be removed. On the other hand, 500 specimens retarded by low temperature for three days, which includes the period during the tests of sterility, were able to remove only 9 grams of tissue.

After five days the amount consumed per hundred maggots was 6 grams; after six days, 5.1 grams; and after seven days, 4.8 grams. Even after eight days over one half of the potential feeding capacity of the maggots was still available. It is interesting to note that even after nine days the therapeutic value of the maggots was still considerable, while with retardation by low temperature the value practically ceased to exist after three days.

On account of the excessive serous exudation from the wound, which is stimulated by the presence of the maggots, a certain amount of necrotic tissue is discharged from the wound with the drainage. This could not be determined by the method used. The data shown, therefore, probably do not represent the maximum amount of tissue removed.

6'. Duration of feeding in the wound. The length of time that surgical maggots are usually allowed to feed in the wound is from three to four days. A few still feed if left for five days. It is shown in Table XI that
nutritionally retarded maggots were able to feed on necrotic tissue for the required period, and this was confirmed by numerous clinical tests. The duration of feeding and the large number that survive under this method increase the therapeutic value of such maggots.

Table XI

Effect of Nutritional Retardation on Length of Feeding Time of Maggots on Necrotic Tissue

<table>
<thead>
<tr>
<th>Retardation Period, Days</th>
<th>Percentage of Maggots Ceasing Feeding after</th>
<th>Two Days</th>
<th>Three Days</th>
<th>Four Days</th>
<th>Five Days</th>
<th>More than Five Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.0</td>
<td>4.2</td>
<td>94.5</td>
<td>0.4</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.0</td>
<td>5.1</td>
<td>91.0</td>
<td>2.5</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.08</td>
<td>14.4</td>
<td>75.4</td>
<td>8.2</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.4</td>
<td>4.1</td>
<td>62.7</td>
<td>22.0</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.2</td>
<td>7.1</td>
<td>29.4</td>
<td>39.0</td>
<td>24.3</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.0</td>
<td>3.1</td>
<td>29.1</td>
<td>39.5</td>
<td>28.3</td>
<td></td>
</tr>
</tbody>
</table>

5'. Application of nutritional retardation.

Both labor and expense in maggot culture can be reduced by nutritional retardation. This method restrains the growth and development of maggots during the sterility tests with the minimum amount of injury. It also permits maggots to
be held for several days after they are ready for use, which eliminates the need of sterilizing eggs daily to maintain a continuous supply of specimens, since sterilization twice a week is sufficient.

After the maggots are received at the hospital the surgeon is frequently unable to use them promptly; it is therefore convenient and economical to be able to hold them over until needed. This can be done when maggots are retarded nutritionally but not when they are retarded by low temperature.

When only a few maggots are required, they are usually purchased from certain pharmaceutical supply houses, and frequently they have to be shipped for long distances. Retardation during transit has been attempted chiefly by packing in ice, but this method has been proved inefficient. Experiments have been conducted on nutritional retardation during transit, not only in the laboratory but in actual practice. Surgical maggots were shipped from Washington, D. C. to New York, Pennsylvania, Ohio, Iowa, North Dakota, Oregon, California, Texas and Alabama. The time spent in transit varied from three to eight days, and in every instance except one* the maggots were reported to be alive and active.

* For some unknown reason the maggots in one bottle were dead on arrival at Ames, Iowa.
on arrival and able to feed for four or five days after being transferred to necrotic tissue. Maggots have also been kept in the laboratory in which this investigation was made for a week or longer and then used in human wounds with excellent results.

When this method of shipping is employed, all the necessary packing is accomplished by simply wrapping the bottle of maggots in a layer of cellulose and inserting it into a carton for mailing. The postage on such a package is comparatively low, the weight being only about one-eighth that of iced packages. The excessive material and labor used in making up the iced package are also rendered unnecessary.

Nutritional retardation, therefore seems applicable to various phases of maggot culture requiring the restrained growth and development of the maggots, and it makes the cultural process more simple, efficient and economical.
IV. CONCLUSIONS

1. Flies of the species *Lucilia sericata* can be reared successfully in a constant temperature cabinet maintained at 26.5° C. with a relative humidity of 40 to 80 per cent.

2. A special type of fly-breeding cage covered with a removable cloth sack instead of wire gauze, and with a top of transparent celluloid is a distinct advantage in fly culture.

3. Raw beefsteak is better than beef heart or beef brain as food for rearing septic brood larvae. The average weight of larvae reared on such food was 46.5 milligrams.

4. The fecundity and longevity of flies is greater when they are fed food consisting of 70 parts water, 30 parts extracted honey, one egg, and one-fourth cake of Fleischmann's yeast per 200 cc. of solution, than when fed either banana, granulated sugar, or milk and honey alone.

5. Clusters of fly eggs may be separated efficiently by the mechanical procedure described in this paper.

6. The egg disinfectant recommended in this paper, namely, 5 per cent formalin plus 1 per cent sodium hydroxide does not cause agglutination of eggs after separation, and
thus has a mechanical advantage over many disinfectants in being able to reach all surfaces of the eggs.

7. This disinfectant gave 100 per cent sterility in routine maggot culture over a period of several years and is thus considered a safe agent to use.

8. The hatch obtained from eggs sterilized with this disinfectant is higher than with nine other disinfectants tried.

9. No disinfectant has been found that will destroy all resistant bacterial spores that conceivably could be encountered, and at the same time not destroy egg viability.

10. Freshly laid fly eggs are not materially damaged by disinfection with the solution recommended, but are injured with the majority of those tried.

11. Retardation of surgical maggots by low temperature is inadequate both in the laboratory and in transit.

12. Retardation by low temperature results in a high mortality (63.3 per cent) even after 24 hours in storage at 5°C to 6°C.

13. Maggots shipped in iced containers seek the warmest portions of the bottle and thus are not subjected to the lowest available temperature.

14. Nutritional insufficiency is an adequate method for maggot retardation.
15. The food described in this paper was found to be a satisfactory retardation-inducing medium.

16. With the use of a retarding food the expense of maggot production is lowered as specimens can be held in the laboratory for a week or more, or shipped long distances without great mortality.

17. Nutritionally retarded maggots are clinically superior to those retarded by low temperature.

18. The remainder of one hundred maggots retarded for five days by the food described can subsequently remove six grams of necrotic tissue, while the same initial number retarded by low temperature can remove only one gram.

19. In conclusion surgical maggots can be produced efficiently and economically by the use of the proper technique in all steps of the breeding and disinfecting process, and by the use of adequate methods of subsequent retardation.
V. SUMMARY

1. Experiments were conducted to devise methods for the culture and transportation of large quantities of vigorous sterile maggots economically.

2. The species of fly used in this work was Lucilia sericata Meigen.

3. A historical account of the therapeutic use of insects is given.

4. A constant temperature rearing cabinet is briefly described, and a blowfly cage is described in detail.

5. An investigation was made of several foods for brood larvae. Beefsteak proved to be the best, and with this food maggots attained an average weight of 46.5 milligrams each. Raw beef from certain other portions of the carcass is apparently as good as steak.

6. A mixture of honey, yeast, egg and water proved to be the most efficient food, of those tried, for adult flies. When this mixture was used the longevity and fecundity were greater than with any of the other foods tested.

7. When flies were fed the honey-yeast mixture, the average number of eggs laid per day per female fly was 55.4, and the average longevity of female flies was 43.8 days.

8. A method of separating eggs from clusters rapidly and without loss or injury was devised. It consists of a
brief softening of the egg clusters between layers of wet cloth, and then separating them by spreading with a spatula.

9. Nine disinfecting combinations were tested on eggs in regard to sterility and mortality produced. A five per cent solution of formalin plus one per cent sodium hydroxide when used for five minutes was found to give most satisfactory results from all standpoints.

10. With the disinfecting technique recommended a 76.8 per cent hatch of fly eggs was obtained.

11. In contrast to most of the disinfectants tried, and to most of those used in the early days of maggot therapy, the one recommended above permits a good hatch when used upon freshly laid eggs.

12. A procedure for sterilizing eggs is suggested which will permit rapid disinfection the same day the eggs are laid, thereby reducing the time involved and lowering the cost of production.

13. A study was made of the value of retardation of surgical maggots by low temperature. The work included an investigation of the proportion of maggots that feed at various temperatures, showing that over 75 per cent of the maggots fed at 10° C.

14. The size of the maggots increased 23.4 per cent when they were stored for seventy-two hours at 10° to 11° C., while at 13° to 14° C. the increase was 90.7 per cent.
Any temperature higher than $12^\circ$ C. was found to stimulate too rapid growth and development for successful retardation of the maggots.

15. The mortality of maggots in storage at low temperatures is extremely high. Even when stored for forty-eight hours at $5^\circ$ to $6^\circ$ C., only one third were able to resume feeding, and the mortality was nearly 100 per cent after six days. This high rate necessitates the early use of the maggots and prohibits their retention for later use when not needed immediately.

16. As this method of retardation has been employed in shipping maggots by mail, its efficiency has been investigated from that standpoint. Tests were conducted under conditions of both summer and winter and it was found that the temperature was not sufficiently low over a long enough period to prevent excessive growth and development during transit.

17. The temperature in a bottle of maggots packed next to ice is not uniform throughout, and maggots migrate to the warmer portions. The retarding effect of the lower temperature is therefore lost.

18. If a sufficiently low temperature could be maintained throughout the period of transit, most of the maggots would be killed, as they are not tolerant of such conditions.

19. A retardation-inducing food, consisting of evap-
orated milk, agar and water, has been devised which elimi-
nates the necessity for the customary method of retardation
by low temperature, both during the sterility tests and
during transit.

20. Tests were conducted to determine the rate of
growth and development during nutritional retardation; it
was found that both processes could be retarded for eight
or nine days without serious injury. This permits surgi-
cal maggots which are not needed immediately to be held for
later use. It also enables long distance shipments to be
made efficiently and with the minimum expense and labor.

21. With beef as a substratum a study was made of
the amount of necrotic tissue removed by maggots after vari-
ous periods of nutritional retardation. This quantity is
vastly greater than when retardation by low temperature is
used. The therapeutic value of the maggots is thus greatly
increased, as removal of necrotic tissue from the wound is
apparently an important factor in the value of this treat-
ment. The amount removed varies inversely, however, with
the length of the period of retardation, owing to the
increased mortality. The period of feeding following retar-
dation is from four to five days, and the maggots have a
tendency to feed slightly longer after an extended period
of retardation than when growth has been arrested for only
a short period.
22. Practical tests were conducted on shipments of nutritionally retarded maggots for long distances. Specimens mailed to various regions of the United States were reported as received in good condition, and as feeding for four or five days after being transferred to necrotic tissue.
VI. LITERATURE CITED


VII. ACKNOWLEDGMENTS

The writer wishes to thank Dr. Elery R. Becker for his patient and understanding counsel throughout the graduate course of study. He also wishes to thank Dr. William Robinson of Washington, D. C. for encouragement and helpful advice.
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Graduate work: Awarded A. M. degree from the George Washington University in 1934. Major work was in Zoology, under the direction of Dr. Paul Bartsch. Admitted to the Graduate School of the Iowa State College in September 1935. Major work for the Ph. D. was taken in Zoology and Entomology under the direction of Dr. Elery R. Becker. Minor work was taken in Veterinary Pathology under Dr. E. A. Benbrook.