THE CULTURE OF SURGICAL MAGGOTS AND CLINICAL ASPECTS OF MAGGOT THERAPY

by

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THE CULTURE OF SURGICAL MAGGOTS
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INTRODUCTION

Since its introduction to surgery by the late Dr. William S. Baer (11), of Johns Hopkins University, maggot therapy has received considerable attention both in this country and abroad. As a result of the widespread popularity of this treatment, many technics for culturing maggots for surgical use have been devised and reported in the medical and biological literature. Because the majority of technics have been too expensive and elaborate for general adoption, the desire for a simpler and less expensive method of culture has been expressed frequently. It was chiefly with the purpose of working out a more practical and economical method of culturing maggots that this investigation was undertaken. It is hoped that the culture methods and the new apparatus herein described will prove helpful not only to those interested in maggot therapy but also to those interested in the general rearing of blowflies or other flies for experimental studies.

In addition, a number of clinical aspects of this problem were carefully studied in order to follow the progress of the various improvements in the application of the treatment, because here too, unless the methods are reasonably simple and inexpensive, the treatment

loses its practicability in spite of its value.

Then too, the writer wished to investigate further the problem of myiasis in wounds, to which two authors (33, 183) had recently called attention. Because these reports appeared contrary to all the experiences with surgical maggots, it was felt desirable to ascertain whether the use of these maggots really constituted a potential source of danger.

Also, in an effort to bring the subject of maggot therapy up to date, a questionnaire was prepared and sent to approximately 125 physicians and investigators who were known to have had considerable experience with this treatment. Through the courtesy of these men, a large amount of information was obtained.

Further, because it was desired to obtain additional information concerning allantoin and urea, substances present in the urinary excretions of the maggot; and since there has been considerable controversy in regard to the use and therapeutic value of these drugs, a number of questions in regard to allantoin and urea were included in the questionnaire mentioned above. Here also, through the courtesy of those who cooperated in the investigation, much information was obtained.

More detailed information, as well as a number of case reports, was secured through personal communications and through several local physicians who cooperated with the writer in this phase of the investigation.

Finally, a study was made of some of the antiseptic and bacteriacidal properties of urea, because these properties of urea are

not well known, and also because there is a dearth of literature on this subject.

HISTORICAL

The observation that maggots are of aid in curing disease may be traced as far back as 1557 when Ambroise Pare, the famous French surgeon, relating his experiences after the battle of Saint Quentin, at la Fere, calls attention to the following:

> The wounds of the injured were very putrid, and full of worms, with gangrene and rottenness so that it was necessary to use the knife to amputate that which was corrupt.....and to correct and arrest the putrefaction, and kill the worms (maggots) which were in the wounds, I washed them with egyptiacum dissolved in wine and brandy.....the cause of making rise up from these (dead) bodies so great a number of big flies which had procreated themselves from the humidity of the dead bodies and the heat of the sun, having their tails (bellies) green and blue, -- that being in the air they made a shadow in the sun.....and I believe that where they settled it would render the air pestilent and cause the plague (65).

He further states:

> .....but mark, after some moneths space, a great number of worms came forth by the holes of the rotten bones from underneath the putrefied Skull; which moved me to hasten the separation and falling away of the putrid bones. I observed three cavities of the largeness of one's thumb filled with wormes about the bigness of points tag.....The bone which nature separated was of the bigness of the palm of one's hand. The patient recovered beyond all mens' expectation (65).

Zachman, in 1704, discussed the origin of maggots in wounds (65).

D. J. Larrey, the celebrated military surgeon of Napoleon's armies during the French campaign in Syria (1799), in his "Memoirs

of Military Surgery", calls attention to the beneficial effects of live fly-maggots in the extensively infected wounds of soldiers.

> During suppuration of wounds, these wounded soldiers were inconvenienced by fly larvae of the blue fly, common in that climate. These insects formed in several hours, developing with such a rapidity that in one or two days they were the size of a small quill. This greatly frightened the soldiers in spite of all efforts to reassure them. It was only after experience that they could be convinced that, far from being injurious to their wounds, these insects accelerated cicatrization by shortening the work of nature and by producing an elimination of the necrotic cells by devouring them. In fact, these larvae only consumed putrid material and did not disturb any living tissues. No hemorrhages were ever observed under these circumstances regardless of the depth to which the insects penetrated or the extent of the wound (65).

J. H. Fabre (1894), in his study of the green bottle fly, observed rapid liquefaction of coagulated albumin in the presence of maggots, and attributed the action to an active pepsin secreted by the larvae (115).

J. F. Zacharias, a military surgeon in the Confederate Army, was perhaps the first person who intentionally employed maggots in wounds. He writes as follows of his Civil War experiences:

> 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 septicaemia, and had rapid recoveries (11).

Goldstein (65), in one of his historical reviews of the use of maggots in wounds, calls attention to the following:

> Surgeons attending the camps and battlefields in time of Aryan supremacy performed amputations, treated injured animals (horses), and perhaps knew of the efficient work of maggots in wounds.

G. Joseph of Breslau (October 18, 1886) discusses 'Uber myiasis externa and interna' and speaks of the 'Maden' (maggots).

Dr. Jacques Munk, of Verebely, (March 21, 1880) discussed 'Uber Maden in Wunden und Hohlen des menschlichen Korpers'.

W. Curran (Medical Press and Circular, pp. 142-144, London, August 25, 1886) recalls a case that occurred under his care at Kussowler in 1869 (medullary cancer of the testicle), and mentions the 'maggots which formed on sores or wounds of every kind in the East is well known'.

George W. Crile, speaking before the Clinical Congress of Surgeons of North America - "War Session", October 23, 1917, said:

> In the wounded who lie out in "No Man's Land" for two or five or ten days, it has been found that the wounds that have done best are those that contain maggots. The reason for this is that there is devitalized tissue; the maggots live on this devitalized tissue, and if they destroy that tissue they do in time what the surgical operation does.

Edward Martin (Philadelphia), in his discussion at the same meeting, said:

> They had been advised by one eminent member of the profession to take all the antiseptics and throw them into the sea, and another had advised them to raise a brood of tame maggots to take care of the wounds.

W. W. Keen (1918), of Philadelphia, says:

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

At the beginning of the twentieth century Larkin, a Chicago surgeon, used maggots in the treatment of osteomyelitis and chronic septic infections, and reported favorable results (190).

Many more references to maggots may be cited, but those mentioned are sufficient to indicate the long and interesting background

these little creatures have had.

In spite of the fact that the beneficial effects of maggots have been noted time and again, Dr. Baer was the first surgeon to put this age-old remedy into use in civil surgery. Let us therefore consider Dr. Baer's observations in greater detail.

BAER'S OBSERVATIONS AND EARLY RESEARCH

The initial impetus for Dr. Baer's subsequent work was an observation he made while he was serving with the American Expeditionary Forces on the battlefields of France. After a battle in 1917 two soldiers with compound fractures of the femur and large flesh wounds of the abdomen and scrotum were brought into the hospital. Although these soldiers had lain on the battlefield for seven days without food or water and had been exposed to the weather and insects of that region, they had no fever and there was no evidence of septicemia. Except for their starvation and thirst, they were in remarkably good condition. This unusual circumstance, in view of the extent of their wounds, and particularly in view of the fact that the mortality of compound fractures of the femur was about 75 to 80 per cent, even when the wounded had the best medical and surgical care that the Army and Navy could provide, attracted Dr. Baer's attention. When he removed the clothing from the wounded parts, he was surprised to find the wounds teeming with thousands of maggots, presumably those of the blowfly. And when the wounds had been irrigated with normal salt solution, he was even more surprised to find that instead of being

filled with pus, which he expected as a result of the degeneration of devitalized tissue and the presence of numerous types of bacteria, the wounds were filled with healthy pink granulation tissue; there was practically no bare bone to be seen; and the inside surface of the injured bone, as well as the surrounding parts, was entirely covered with the same pink granulation tissue which filled the wounds. Bacterial cultures showed a few staphylococci and streptococci, but not enough to cause pus formation. The maggots had accomplished their task – the wounds had healed.

What Dr. Baer saw on the battlefield made a deep impression on him. After thinking about this experience for ten years, he decided to put his observations into practical use in civil surgery. In September, 1929, four children suffering from chronic osteomyelitis were admitted to the Children's Hospital in Baltimore. Each child had previously undergone operation three or four times and had been treated over a period of from one to five years. On these children, who had been unsuccessfully treated by the usual methods, Dr. Baer began his first work in the treatment of osteomyelitis with maggots. First all the dead tissue was thoroughly removed from the affected areas. Then maggots of the blowfly, obtained from the immediate neighborhood, were placed without sterilization into the wounds. The maggots were replaced several times. At the end of about six weeks, the wounds had healed completely – the deeper structures as well as the skin.

The experimental treatments were continued. In the early treatments, however, difficulties arose from secondary infections. In

three instances gas bacilli were discovered in the wounds. Although the patients presented no clinical symptoms of gas gangrene, all treatments were discontinued and research begun to solve the problem. From the experiments conducted, Dr. Baer was able to show that maggots destroyed rather than caused gas bacilli infection. And he concluded that in order to overcome gas bacilli it would merely be necessary to increase the quantity of maggots, after first making sure that the maggots placed into the wounds were free from all gas bacilli.

Following these findings, the treatment of wounds with maggots was resumed. But it was not long before tetanus (lockjaw) bacilli were found in the wounds of some of the patients. These wounds were quickly washed out and the patients were given injections of tetanus antitoxin. Four patients manifested no clinical symptoms; two developed severe lockjaw; one, who had been suffering from advanced tuberculosis of the ankle, lung and spine, died in spite of the administration of large doses of antitoxin; and the last patient finally recovered after a long struggle with the infection.

Such disastrous experiences made Dr. Baer realize that in civil practice it would be necessary to use sterile maggots. In order to have a supply of such maggots on hand throughout the year, flies would have to be grown successfully in the laboratory. After extended research a successful technic was devised for producing the flies and culturing sterile maggots.

Dr. Baer continued with his maggot treatment of chronic osteomyelitis until April, 1931, when he suddenly died. Fortunately for

medicine, he had observed and had treated a large number of patients, and had been able to report 95 per cent cures in children and 85 per cent cures in adults. So excellent were the results he obtained that it was not long before many other surgeons adopted his method of maggot therapy. A recent report (164), to be discussed subsequently, shows that the treatment has been given in every state in the United States as well as in Canada and a large number of foreign countries.

SPECIES OF LARVAE USED IN MAGGOT THERAPY

The larvae used in maggot therapy are those which are known to feed upon decaying and necrotic tissue. Satisfactory larvae are obtained from the bronze-green blowflies, Lucilia sericata (Meigen) and Lucilia caesar (Linn.); the blue-black bottlefly, Phormia regina Meigen; and the large blue-bottle-flies Calliphora erythrocephala (Meigen), Calliphora vomitans (Linn.) and Cynomyia cadaverina Desv. Of the flies used, Lucilia sericata and Lucilia caesar are the most satisfactory because of the ease with which they can be handled in the laboratory. Great care must be exercised to identify correctly the species of fly to be used, for the larvae of some species of flies are unable to distinguish between dead and living tissue and will feed as voraciously upon healthy tissue as upon dead tissue. The screw-worm flies Cochliomyia macellaria (Fabr.) and Cochliomyia americana Cushing and Patton are well known examples of this.

MYIASIS

Recently Chandler (33) and Stewart (183) have called attention to the fact that the blowfly, Lucilia sericata, with a few other species of flies which have been used widely in treating osteomyelitis and other suppurative infections in man, will on occasion attack healthy tissue when necrotic tissue is not available. Chandler also called attention to the fact that Lucilia sericata is known to cause terrible myiasis of man in China and states that Stewart (183) has demonstrated that though this species shows a distinct preference for necrotic tissue it would willingly attack healthy tissue, and even tear a hole through normal skin. Because these reports appeared contrary to all the experiences with surgical maggots, it was deemed desirable to investigate further concerning the problem in an effort to ascertain whether the use of these maggots really constituted such a potential source of danger. First, the literature was reviewed in order to find any additional information of a similar nature. Secondly, the writer communicated directly with Dr. Stewart in regard to this matter, as well as with Dr. William Robinson, Senior Entomologist of the United States Department of Agriculture, Division of Insects Affecting Man and Animals, who has had considerable experience with maggot therapy. And finally, the question, "have you ever experienced myiasis?" was included in the questionnaire referred to above, in the hope that this would afford an additional source of information.

As a result of reviewing the literature several articles dealing with myiasis were found in addition to the one published by Stewart (183). These were by Brumpt (23), Patton and Evans (140), Messer and McClellan (124), and Hobson (85).

In reviewing Stewart's article (183), the following information was found of interest.

> During the course of clinical investigations, the writer saw several cases of osteomyelitis being treated with maggots in which there was unmistakable evidence that normal vital tissue was being attacked by these organisms. One case in particular was that of a girl, 13 years of age, with an osteomyelitis of the lower third of the tibia. After several implantations of L. sericata larvae, nearly three-fourths of the circumference of the ankle was denuded sufficiently to expose the bones and tendons; and yet from X-ray and clinical examinations it was determined that not much more than one-quarter of this area was occupied by necrotic or infected soft tissue.
>
> Patton and Evans (1929) report that larvae of this same species of blow-fly produce terrible myiasis, often accompanied by great destruction of tissues, in man in China.
>
> In an attempt to reproduce what was seen in the above-mentioned clinical investigations, the writer ventured to create experimental myiasis in laboratory animals, i.e. guinea-pigs. A piece of skin, extending down to the muscle tissue, was removed under strictly aseptic conditions from the dorso-lateral aspect of the left hip of each of five guinea-pigs. A considerable area around each wound was shaved so that the fur of the animals would not prevent the maggots from escaping from the surgical wounds if they so desired. Immediately after the excisions, 48-hour sterile Lucilia sericata larvae from a breeding stock generously supplied by Dr. F. C. Bishopp and Dr. G. F. White, of the U. S. Bureau of Entomology, were introduced into the wounds. In every case the maggots established themselves as rapidly in the sterile wounds, devoid of necrotic tissue, as they would in an osteomyelitis wound. In each pig, deep extensive multiple sinuses were formed by the larvae within a few hours. In one animal the peritoneum was penetrated and viable maggots were recovered from the abdominal cavity.

The maggots almost invariably worked away from the excised area into the muscle underlying the surrounding epidermis. Death resulted to the experimental animals without exception. Maggots were also introduced into dry wounds, and while they always succeeded in establishing themselves and created sinus tracts it was much more difficult for them to do so when the wounds were moist.

Following this series of experiments, sterile L. sericata larvae were introduced into a long-established human osteomyelitis wound of the lower tibia, in which there was plenty of necrotic tissue, both soft and osseous, though it was not immediately accessible. The maggots established themselves very quickly and 24 hours later had destroyed an appreciable quantity of normal tissue. Seventy-two hours after implantation, so much normal tissue had been attacked and so much damage had been done that the larvae had to be removed from the wound; little if any necrotic tissue had been ingested. It becomes perfectly apparent from these experiments that the larvae of L. sericata can, and will, establish themselves in and feed upon normal healthy tissue.

Brumpt (1933) stated his belief that there are two biological races of Lucilia sericata, one which produces larvae that feed exclusively upon necrotic tissue and can therefore be utilized in surgical practice, and another which produces larvae which are capable of destroying healthy tissue and cannot be surgically employed. With this thought in mind, a breeding-stock of L. sericata was secured through the kind cooperation of Dr. Roy Melvin of the U. S. Entomological Laboratory in Dallas, Texas, from specimens originally collected in that city, and therefore of quite different origin from the material supplied by Dr. Bishopp and Dr. White. Larvae from this stock were introduced into guinea-pigs in exactly the same way as were the first stock, with identical results. Consequently, Dr. Brumpt's belief that possibly the French and Chinese strains differ in feeding habits from the American strain must be discarded.

It has been observed in clinical experience that, when small or moderate numbers of L. sericata larvae were introduced into osteomyelitis wounds where there was an abundance of necrotic tissue present, the vital tissue was not attacked. This indicates a preference on the part of the maggots for dead tissue, and an attempt was made to determine the accuracy of such a supposition. On the dorso-lateral aspect of the left hip of each of four guinea-pigs a piece of skin, extending down to the muscle tissue, was removed, after first shaving the entire hip, and a pure virulent 24-hour culture

of Staphylococcus aureus was introduced. As soon as these wounds had become infected and necrotic tissue was apparent, similar but aseptic excisions of equal dimensions were effected in the shaven areas. This second series of excisions were separated from those of the first series by bridges about half a centimetre wide covered by intact epidermis. These new wounds were made as attractive as possible by finely shredding some of the muscle tissue. As soon as the second excisions were made, sterile 48-hour Lucilia sericata larvae were introduced into the uninfected wounds. In every instance the maggots crossed the intervening strip of intact epidermis and localized in the necrotic tissue of the infected wound within ten minutes' time, thereby demonstrating their preference for necrotic tissue. In a single guinea-pig, two larvae remained crawling about on the surface of the uninfected wound for several hours, but they were not observed to feed thereon.

Hobson (85) writes: There appears to be no published evidence as to the ability of these larvae (Lucilia and Calliphora species) to penetrate membranes; however, their mouthparts resemble those of the Congo floor maggot, Auchmeromyia luteola. This blood-sucking larva can readily bore through human skin.

Phormia regina, Wohlfartia nuba and Lucilia caesar are three other species of blow-flies whose larvae are used more or less commonly in the treatment of osteomyelitis, and it is probable that they are quite as potentially dangerous as are the larvae of L. sericata. The habits of L. caesar are so close to those of L. sericata that we are probably safe in assuming that they too will attack vital tissue if necrotic tissue is not immediately accessible.

Stewart concludes as follows:

L. sericata maggots will attack vital normal tissue unless necrotic tissue is immediately available, and they can penetrate intact healthy epidermis. They prefer necrotic tissue, however.

Whereas these organisms play an important therapeutic role, they must be utilized with care by an experienced individual.

In contrast to Stewart's work the following information is significant. Messer and McClellan (124) state as follows:

> Hobson has carried out an investigation of the digestive enzymes of Lucilia sericata larvae, and has found a trypsin-like protease in both the digestive tract and excreta (83), and a collagenase in the excreta (82). In England, this larvae is said to infest sheep, boring into the flesh through the skin. The American variety of the same species, however, was chosen for surgical purposes, because here it is known never to attack living tissue. We have studied the digestive enzymes of L. sericata to learn if such a difference in food habits involved a corresponding difference in enzyme mechanism. We were not able to identify a collagenase in the solution of excreta, although Hobson appears to have found one in that variety of L. sericata common in England. Since such an enzyme would aid the larva to penetrate through the intact skin of an animal, we believe that this difference in enzyme equipment is a reflection of the difference in feeding habits between the English variety of L. sericata and that found in America.

In reply to the communication with Dr. Robinson referred to above in regard to myiasis, Robinson stated:

> It is true that reports show that this species (Lucilia sericata) causes injury to sheep. I am not so sure that the reports of injury to man in China can really refer to this species, because here in America the occurrence of the maggots in a wound is associated with remarkable healing effects. We have never seen a report or heard of one in connection with maggot therapy here in which any injury was caused by maggots preferring living tissue to necrotic material in a wound.

The results of the questionnaire showed that only two physicians experienced myiasis, and in order to inquire more fully regarding the extent to which myiasis had occurred in their work, the writer again communicated with these men. Only one of the physicians replied, and in regard to the matter he states:

> Myiasis occurred once when some "screw flies" inadvertently got into the stock. They were removed from the stock, and this trouble has never again occurred.

This physician reported having treated 300 cases of chronic osteomyelitis. Myiasis in this case was attributed to the screw-worm

fly, a species which is definitely known to cause myiasis.

It was also of interest to note the results of some of the other physicians regarding myiasis. One physician reported having treated 1,500 cases of acute and chronic osteomyelitis, as well as indolent ulcers, but never experienced any myiasis. Two other physicians reported having treated approximately 1,000 cases of acute and chronic osteomyelitis, malignant ulcers, carbuncles, varicose ulcers, and staphylococci and streptococci infections without having experienced any myiasis. Still another physician reported having treated about 400 cases of chronic and acute osteomyelitis, but never noticed any myiasis. Similar results were reported by the majority of the physicians.

The literature likewise contains hundreds of case reports from the treatment with maggot therapy, but to the writer's knowledge no one has ever reported having experienced myiasis, or of ever having had any ill effects with this treatment, other than the investigators referred to above.

This does not mean, of course, that myiasis has not occurred, or can never occur. The writer feels that the environmental factors under which maggots are utilized are important, and under certain unfavorable conditions such as overcrowding or absence of necrotic tissue, it may be entirely possible to force a harmless species of maggot to harmful activity.

Stewart's conclusions in regard to Brumpt's belief of the differences in feeding habits of the Chinese, French, and American

strains of Lucilia sericata do not appear entirely justified on the basis of the amount of work which this investigator has done. It may be entirely possible that differences in feeding habits are exhibited among the American strains of Lucilia sericata, and if such were the case these differences of opinion could be readily understood. However, until more work is done, the results of Stewart's investigation can not be interpreted too positively.

The writer is entirely in accord with Stewart when he states that maggots must be utilized with care by an experienced individual, but after all, what therapeutic measure should not be? A drug in the hands of the inexperienced may prove infinitely more hazardous than the careless application of surgical maggots.

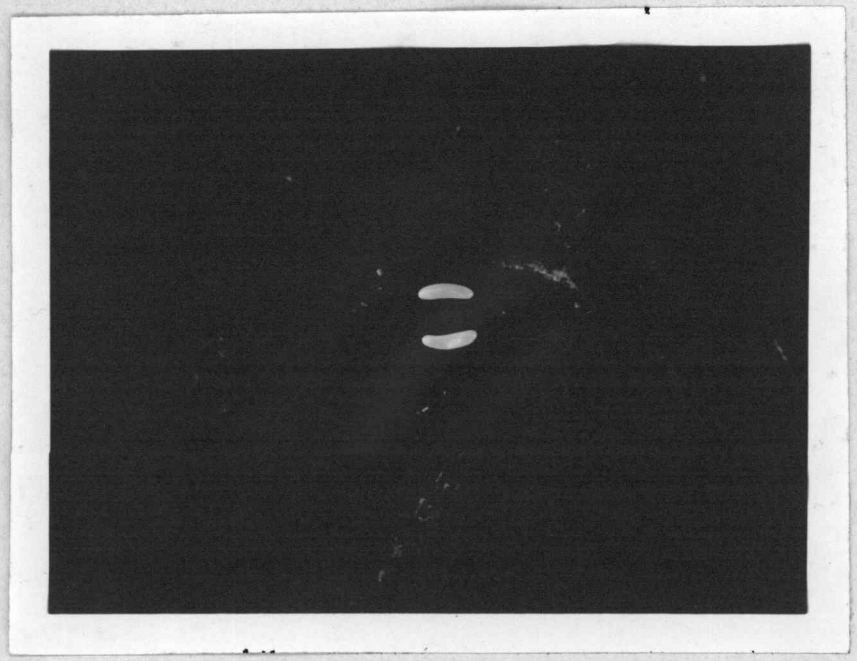
On the basis of the evidence presented here, it is concluded that myiasis, although possibly a potential source of danger in maggot therapy, has not thus far presented a serious problem. Clinical experience has corroborated this fact.

LIFE HISTORY OF THE FLY

There are four stages in the life history of the blowfly, namely: (1) the egg, (2) the larva (maggot), (3) the pupa, and (4) the fly. Plates 1, 2, and 3.

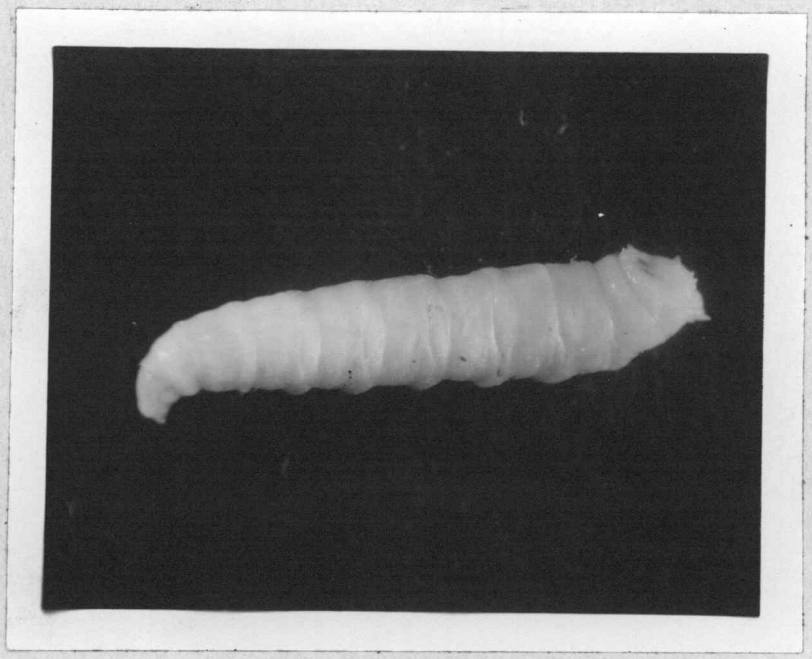
The Egg

The egg is white, slightly curved, and extremely small, measuring approximately 0.5 by 1.5 mm. The eggs are laid in clusters

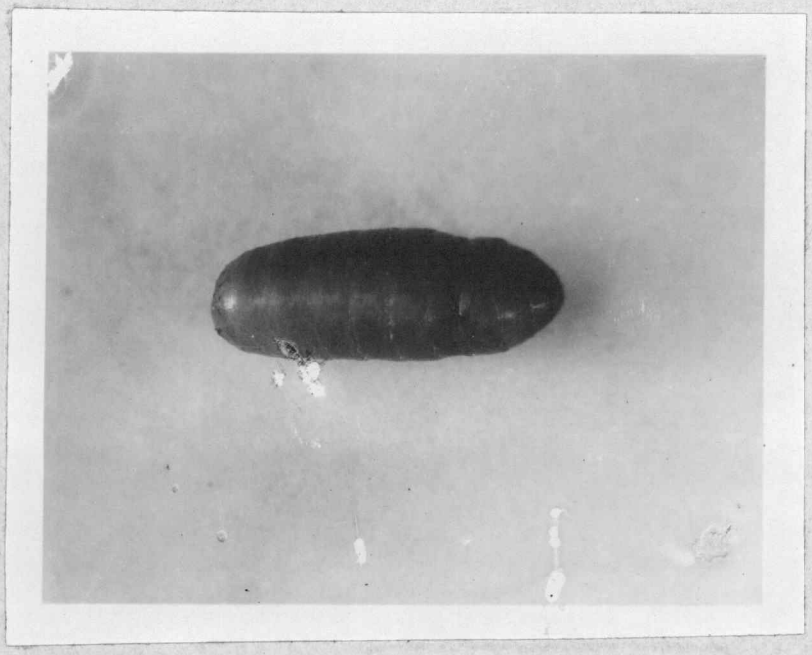


The Eggs

Plate 1

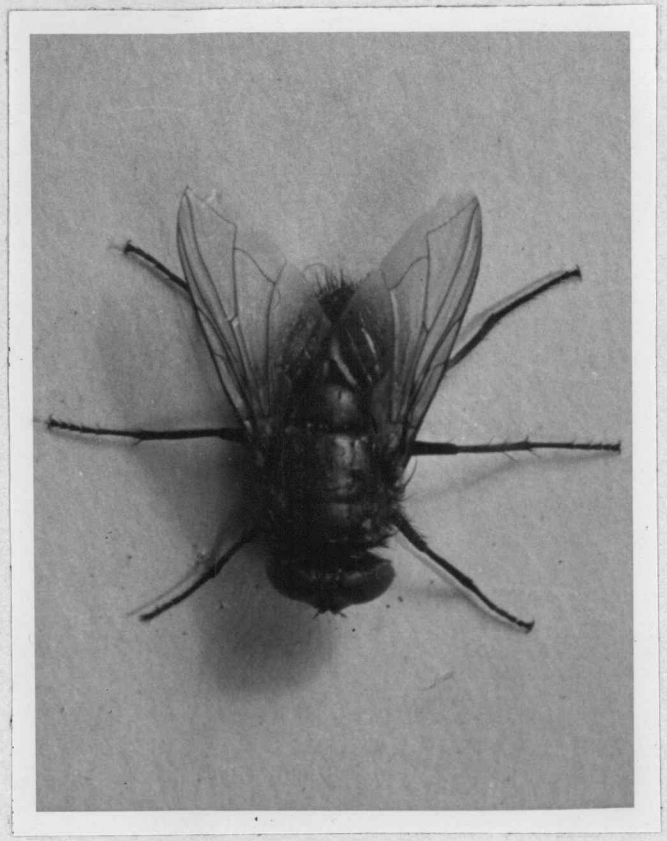


The Larva

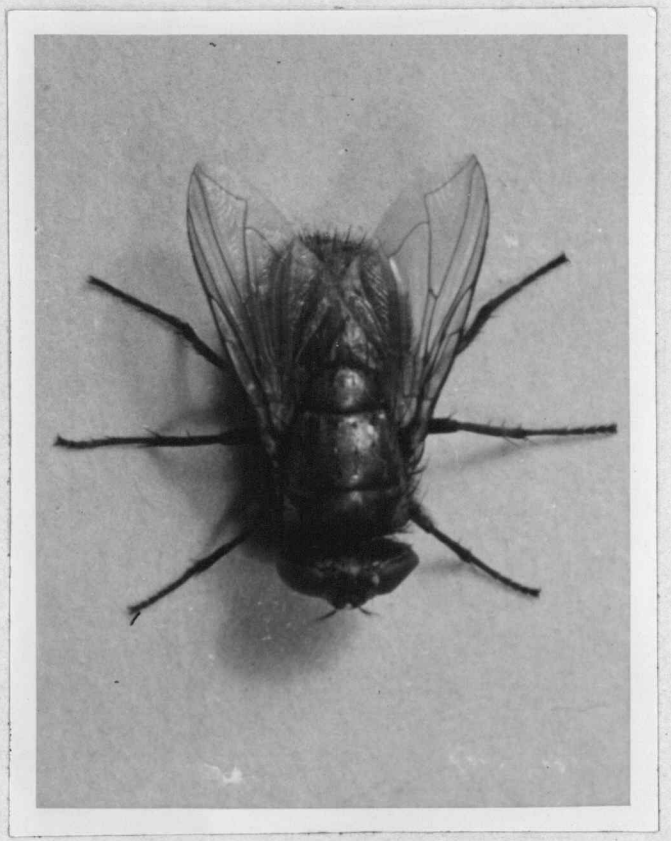


The Pupa

Plate 2



Male



Female

The Flies

Plate 3

upon such matter as meat, fish, carrion, or any other substance which possesses the proper chemotropic quality. It takes from 8 to 24 hours for the eggs to hatch.

The Larva (Maggot)

The larval stage consists of two periods: (1) the active feeding and growing period, generally from 4 to 7 days, during which the larvae feed voraciously and are of surgical use; and (2) the prepupal or inactive period, usually from 2 to 6 days, during which the larvae do not feed and are useless for surgical purposes. In the prepupal period the larvae are found moving about on the surface of the pupation medium,[1] or are observed making their way into the medium to pupate. The larvae at this stage exhibit an unusually intense activity and are capable of squeezing into and out of very narrow places, hence, if the larvae are not closely confined, they will escape. Larvae will migrate from their food prematurely and try to escape if gases formed during the feeding period are allowed to accumulate or if too many larvae are confined to one container. If insufficient food is given, many of the larvae will also migrate prematurely. In these cases the larvae will develop into small flies with low egg-laying capacity. Therefore, some degree of care should be exercised to obviate these factors. Larvae have no eyes and yet are repelled by light. Breathing is accomplished mainly through two spiracles in the enlarged

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[1]Most commonly sand. Sawdust, wood chips, gauze, coiled cloth and dry boxes are frequently used.

posterior extremity of the body. A sufficient supply of oxygen is necessary, otherwise mortality results. The larvae ingest only liquid or semi-liquid food, since they possess no teeth. Stewart (183) has shown that blowfly larvae are able to digest necrotic tissue by the macerating activities of the mouth-hooks and by predigestion effected by tryptase present in the excreta, which is active in an alkaline medium. Such alkalinity is effected by ammonia in the excreta and by calcium carbonate exuded from the body walls of the maggots. Slocum, et al (177) have shown that the enzymes in the excreta are only weakly proteolytic. The strong enzyme is present within the digestive tract of the maggot where it completes digestion. Light is not essential for the growth of the larvae and they feed better in dark places.

The Pupa

Toward the end of the prepupal period the larvae become quiescent, begin to shorten, and assume an oval form. Their outer skin begins to harden and changes in color; finally they metamorphose into the pupa which is deep dark brown in color. The pupal stage generally lasts from 6 to 10 days.

The Fly

Emergence of the fly lasts for several days. Males emerge first, then males and females, and finally only females appear. The fly emerges from its pupal case by breaking off the operculum,

or pupal cap. This is accomplished by the distension of the frontal sac, a large, bladder-like organ located on the fly's forehead. Upon first emerging, the fly is dusty-gray in color, extremely soft, and easily crushed. The wings are crumpled and vestigial in appearance, but stretch out in about 2 to 3 hours to their fully developed form. The flies change in color from a dusty-gray to a darker gray with a marked purplish tinge, and then, finally to a distinct bronze-green. The females are readily distinguished from the males by the greater distance between their compound eyes. The females depend for sexual development upon an adequate diet. This must consist of some protein food such as liver or beef. Mackerras (108) showed that female flies did not become sexually mature until they had fed once on an adequate protein diet. This is not necessary in the case of the male flies. Johnston and Tiegs (93) noted that male blowflies become sexually mature soon after emergence, and Cowan (37) has also made similar observations. Oviposition generally takes place within 10 days after the flies emerge, but preoviposition may take as long as 20 days. The number of eggs laid daily by one fly varies considerably, but usually averages around 100. The flies continue to lay eggs well for about 3 to 4 weeks, after which time egg production and viability of eggs decrease. Under laboratory conditions the life cycle of the fly is about 25 to 30 days.

ENVIRONMENTAL FACTORS

Some form of incubation for the purpose of maintaining a uniform temperature during the brood production of flies, as well as during the culture of sterile maggots, has been universally recommended. Since any form of incubation involves some expense, it was felt desirable to investigate the possibility of culturing both maggots and flies entirely in the laboratory without incubation, and to note whether the rate of growth in the incubator under constant temperature (78° to 80° F.) and humidity (50 to 60 per cent) was necessarily more regular than the rate of growth in the laboratory under fluctuating temperature (50° to 95° F.) and humidity (35 to 75 per cent).

It also was desired to find out whether the mortality of the flies grown in the laboratory tended to increase, or if any marked changes in the general character of eggs, larvae, pupae and flies were manifested as compared with the eggs, larvae, pupae and flies grown in the incubator.

Moreover, attention was given to the factors of egg-laying capacity, egg viability, and mortality rates in order to determine whether the flies grown in the laboratory showed any marked differences in these respects when compared with the flies produced in the incubator.

Most of these problems arose from the fact that when Dr. Baer began his researches, he found it impossible to keep his flies alive in the laboratory for more than a few days after emergence from the pupal

stage. This led him to the conclusion that perhaps the laboratory air did not contain sufficient moisture, the temperature was not constant, or the food was insufficient. It was then that he and his assistants devised the elaborate method of culture which has been adopted by many using maggot therapy. Special incubators for both flies and maggots were constructed in which the temperature and humidity were controlled, artificial light supplied, and circulation of air carried on by means of small motors. The fact that flies could not be kept alive in the laboratory appeared rather surprising to me considering the varied conditions under which flies thrive normally in nature. Since Dr. Baer's technic was worked out, even further improvements have been added. Recently, however, the tendency has been toward simplification of technic rather than complexity, but many of the modified methods are still too elaborate. In view of this fact, it was felt desirable to ascertain whether growth of the entire life cycle of the fly could be carried on successfully under ordinary laboratory conditions, and if so, what conditions are necessary to maintain a healthy strain of both maggots and flies.

Bearing in mind, however, the difficulties of culture reported in the literature, I also undertook the problem of devising a method of incubation which could be made as economical as possible, and to study the advantages, if any, over the former method. This phase of the study, therefore, was developed as a comparison of growth in the laboratory with growth in the incubator.

Four fly cages were used in this study, marked Cages 1, 2, 3, and 4, respectively. Cages 1 and 2 were placed on a table in a well lighted laboratory room that measured approximately 27 feet long, 27 feet wide, and 14 feet high. Light entered the room from three large windows on the east side and from a similar number of windows on the north side. Heat was supplied by three large steam radiators. The cages were thus surrounded by an abundance of natural light and heat.

Cages 3 and 4 were placed into a medium-sized brood incubator which was regulated for temperature at about 78° to 80° F., and a relative humidity of between 50 to 60 per cent. Cage 3 was placed on the middle shelf of the incubator where an abundance of light was afforded. Cage 4 was placed on the lower shelf of the incubator where the supply of light was poor, because the glass sides in this section of the incubator were heavily coated with paint. It was desired to determine whether a poorly illuminated environment had any effect on mortality rate, egg-laying capacity, egg viability, or the general character of the flies. The incubator was kept in the same laboratory room as the fly cages.

A summary of the results of this study, representing six life cycles, is given in Tables 1, 2, 3, and 4. These results are briefly as follows:

1. The growth of all of the stages in the life cycle of the fly can be carried on entirely in the laboratory, and the flies can lay eggs and live in the laboratory as well as in the incubator.

2. The rate of growth in the incubator under a constant temperature (78° to 80° F.) and humidity (50 to 60 per cent), was no more regular than the rate of growth in the laboratory under fluctuating temperature (50° to 95° F.) and humidity (35 to 75 per cent).

3. There were no noteworthy differences manifested either in fly mortality, egg-laying capacity, egg viability, or the general character of the eggs, larvae, pupae, and flies grown in the laboratory as compared with those reared in the incubator.

4. As far as illumination was concerned, no noteworthy differences were observed in the flies exposed to a poor or well illuminated environment regarding fly mortality, egg-laying capacity, egg viability, or general character of the flies.

In laboratories where the insulation is quite good, and where the temperature does not fluctuate too greatly and the humidity does not fall too low, special incubators for the housing of flies and maggots is unnecessary. The culture process may be carried on entirely in the laboratory. If, however, development should fall below a certain desired rate, growth may be hastened by means of incubation. This need not involve any great expense. A very efficient and economical type of incubator which the writer has found highly satisfactory in his work is described in the section on apparatus for brood production. An even simpler form of incubation which was found quite satisfactory consists of placing over the fly cages or brood

LEGEND

Cages 1 and 2 represent those kept in the laboratory; Cages 3 and 4 those kept in the incubator.

TABLE 1. SUMMARY OF FLY MORTALITY, GENERATIONS 1-2-3-4-5-6

Gen. No.	Cage No.	Days	Dead	Nor.	Abnor.	Inj.	Small	Medium	Large	Males	Females
1	1	36	13	12	1	7	8	3	2	8	5
1	2	36	13	10	5	4	5	4	4	7	6
1	3	39	10	5	5	3	4	3	3	6	4
1	4	41	15	11	4	5	8	5	2	8	7
2	1	27	8	4	5	1	4	3	1	5	3
2	2	26	7	4	3	1	4	2	1	4	3
2	3	27	12	4	6	2	6	2	4	5	7
2	4	30	13	11	1	4	6	1	6	7	6
3	1	28	16	15	2	3	7	7	2	8	8
3	2	30	5	4	1	1	2	1	2	2	3
3	3	30	7	5	2	3	4	2	1	2	5
3	4	28	14	12	2	5	7	5	2	9	5
4	1	31	24	23	1	9	4	11	9	14	10
4	2	30	17	16	2	7	4	5	8	7	10
4	3	33	11	9	2	4	5	4	2	6	5
4	4	34	26	13	2	6	13	6	7	20	6
5	1	23	9	8	3	5	1	6	2	7	2
5	2	25	12	10	2	2	2	4	6	5	7
5	3	29	18	17	4	6	3	7	8	10	8
5	4	26	8	6	2	2	2	2	4	3	5
6	1	23	13	7	2	1	3	4	6	5	8
6	2	27	9	8	1	4	2	3	4	5	4
6	3	24	10	7	1	3	3	3	4	7	3
6	4	26	11	6	3	4	4	1	6	3	8

TABLE 2. SUMMARY OF THE LIFE CYCLES OF GENERATIONS 1-2-3-4-5-6

Gen. No.	Cage No.	New gen. started. Date	Size of Flies Small	Med.	Large	Condition of Flies Normal	Abnor.	Oviposition Began Date	Days since emerging from pupa.	Time for eggs to hatch. Hours	Feeding period of maggots. Days	Size of maggots at time of entrance to pupation medium mm.	Character of pupation medium	Prepupal period Date Began	Ended	Pupation period Date Began	Ended	per cent pupal hatch	Total period of the life cycle. Days
1	1	10-23-7	/	//	//	///	(/)	11-11-7	19	8-10	5	15-16	sand	11-16-7 11-16-7	11-17-7 11-20-7 (1-4d.)	11-17-7 11-17-7	11-29-7 11-30-7 (12-13d.)	98	37
1	2	10-23-7	/	/	///	///	(/)	11-10-7	18	8-10	5	15-16	sand	11-16-7 11-16-7	11-16-7 11-22-7 (2-6d.)	11-16-7 11-16-7	11-29-7 11-30-7 (11-13d.)	100	37
1	3	10-23-7	/	/	///	///	(/)	11-11-7	19	16-24	5-6	15-16	sand	11-16-7 11-16-7	11-23-7 11-24-7 (5-6d.)	11-23-7 11-23-7	12-2-7 12-4-7 (9-11d.)	100	40
1	4	10-23-7	/	//	//	///	(/)	11-12-7	20	20-24	5	15-16	sand	11-16-7 11-16-7	11-23-7 11-24-7 (5-6d.)	11-23-7 11-23-7	12-4-7 12-6-7 (11-13d.)	97	42
2	1	11-29-7	/	//	//	///	(/)	12-10-7	11	8-10	5	15-16	sand	12-16-7 12-16-7	12-18-7 12-22-7 (2-6d.)	12-16-7 12-16-7	12-27-7 12-29-7 (9-11d.)	100	28
2	2	11-29-7	/	/	///	///	(/)	12-9-7	10	10-12	5	15-16	sand	12-15-7 12-15-7	12-15-7 12-20-7 (4-6d.)	12-15-7 12-19-7	12-26-7 12-29-7 (7-10d.)	96	27
2	3	12-2-7	/	/	//	///	(/)	12-14-7	12	10-15	5	15-16	sand	12-20-7 12-20-7	12-23-7 12-25-7 (3-5d.)	12-23-7 12-23-7	12-29-7 12-30-7 (6-7d.)	99	28
2	4	12-4-7	/	/	///	///	(/)	12-15-7	14	12-18	5	15-16	sand	12-24-7 12-24-7	12-27-7 12-28-7 (3-4d.)	12-27-7 12-27-7	1-4-8 1-6-8 (8-10d.)	100	31
3	1	12-27-7	/	///	/	///	(/)	1-5-8	9	10-12	5-6	15-15	sand	1-12-8 1-12-8	1-17-8 1-18-8 (5-6d.)	1-17-8 1-17-8	1-25-8 1-27-8 (8-10d.)	98	29
3	2	12-28-7	/	/	///	///	(/)	1-2-8	7	10-12	4	15-16	sand	1-7-8 1-7-8	1-10-8 1-12-8 (3-5d.)	1-10-8 1-10-8	1-26-8 1-28-8 (15-18d.)	100	31
3	3	12-29-7	/	/	///	///	(/)	1-13-8	15	8-10	5	15-16	sand	1-19-8 1-19-8	1-23-8 1-24-8 (4-5d.)	1-23-8 1-23-8	1-30-8 2-2-8 (7-10d.)	100	32
3	4	1-4-8	/	//	//	///	(/)	1-18-8	12	10-15	5	15-16	sand	1-22-8 1-22-8	1-25-8 1-26-8 (3-4d.)	1-26-8 1-26-8	2-2-8 2-4-8 (8-10d.)	99	29
4	1	1-25-8	//	/	//	///	(/)	2-12-8	18	8-10	4	15-16	sand	2-17-8 2-17-8	2-19-8 2-20-8 (2-3d.)	2-19-8 2-19-8	2-28-8 2-28-8 (7-9d.)	100	32
4	2	1-26-8	/	/	///	///	(/)	2-12-8	17	8-10	4-5	15-16	sand	2-17-8 2-17-8	2-20-8 2-21-8 (3-4d.)	2-20-8 2-20-8	2-26-8 3-2-8 (6-10d.)	97	31
4	3	1-30-8	/	/	///	///		2-12-8	13	8-10	5	15-16	sand	2-18-8 2-18-8	2-20-8 2-22-8 (2-4d.)	2-20-8 2-20-8	3-5-8 3-7-8 (12-14d.)	98	34
4	4	2-2-8	//	/	//	///		2-16-8	14	20-24	4	15-16	sand	2-21-8 2-21-8	2-22-8 2-23-8 (1-2d.)	2-22-8 2-22-8	3-2-8 3-2-8 (10d.)	100	35
5	1	2-26-8	/	/	///	///	(/)	3-6-8	8	15-20	4	15-16	sand	3-11-8 3-11-8	3-13-8 3-14-8 (2-3d.)	3-13-8 3-13-8	3-22-8 3-24-8 (9-11d.)	97	24
5	2	2-28-8	/	/	///	///	(/)	3-9-8	11	10-12	5	15-16	sand	3-15-8 3-15-8	3-16-8 3-17-8 (1-2d.)	3-16-8 3-16-8	3-24-8 3-27-8 (8-11d.)	100	26
5	3	3-5-8	/	/	///	///	(/)	3-13-8	10	12-18	5	15-16	sand	3-21-8 3-21-8	3-24-8 3-25-8 (3-4d.)	3-24-8 3-24-8	4-4-8 4-7-8 (11-14d.)	98	30
5	4	3-9-8	/	//	//	///	(/)	3-21-8	12	10-15	4	15-16	sand	3-26-8 3-26-8	3-28-8 3-30-8	3-28-8 3-28-8	4-5-8 4-6-8 (8-9d.)	99	27
6	1	3-22-8	/	/	///	///	(/)	3-30-8	8	8-10	5	15-15	sand	4-5-8 4-5-8	4-7-8 4-8-8 (2-3d.)	4-7-8 4-7-8	4-14-8 4-13-8 (7-9d.)	100	23
6	2	3-24-8	//	/	//	///	(/)	4-4-8	11	10-12	4	15-15	sand	4-9-8 4-9-8	4-12-8 4-14-8 (3-5d.)	4-12-8 4-12-8	4-20-8 4-22-8 (8-10d.)	100	27
6	3	4-4-8	/	//	//	///	(/)	4-14-8	10	8-10	4	15-15	sand	4-19-8 4-19-8	4-20-8 4-22-8 (1-3d.)	4-20-8 4-20-8	4-28-8 4-30-8 (8-10d.)	100	24
6	4	4-5-8	/	/	///	///	(/)	4-17-8	12	15-20	5	15-15	sand	4-23-8 4-23-8	4-26-8 4-27-8 (3-4d.)	4-26-8 4-26-8	5-2-8 5-5-8 (6-9d.)	97	27

TABLE 3. RESULTS OF EXPERIMENT TO DETERMINE WHETHER EGGS, LARVAE, AND PUPAE CAN BE GROWN SUCCESSFULLY UNDER LABORATORY CONDITIONS WITHOUT CONTROLLED TEMPERATURE AND HUMIDITY

Expt. No.	Larval container No.	Life cycle began. Date	Time for eggs to hatch. Hours	Feeding period of maggots Days	Size of maggots at time of entrance to pupation medium. mm.	Character of pupation medium.	Prepupal period Date Began	Prepupal period Date Ended	Pupation period Date Began	Pupation period Date Ended	per cent pupal hatch	Size of Flies Small	Size of Flies Med.	Size of Flies Large	Condition of Flies Nor.	Condition of Flies Abnor.	Oviposition Began Date	Oviposition Days since emerging from pupa	Total period of the life cycle Days
1	3	11-11-7	18-24	5-6	15-16	sand	11-18-7 11-18-7	11-23-7 11-24-7 (6-6d.)	11-23-7 11-23-7	12-2-7 12-4-7 (9-11d.)	100	♂♂	♂	♀♀	♀♀♀	(♂)	12-14-7	12	33
1	4	11-12-7	20-24	5	15-16	sand	11-18-7 11-18-7	11-23-7 11-24-7 (6-8d.)	11-23-7 11-23-7	12-4-7 12-6-7 (11-13d.)	97	♂	♂	♀♀♀	♀♀♀	(♂)	12-13-7	14	36
2	3	12-14-7	10-15	5	15-16	sand	12-20-7 12-20-7	12-23-7 12-25-7 (3-5d.)	12-23-7 12-23-7	12-29-7 12-30-7 (6-7d.)	99	♂	♂	♀♀♀	♀♀♀	(♂)	1-13-8	15	30
2	4	12-18-7	12-18	5	15-16	sand	12-24-7 12-24-7	12-27-7 12-28-7 (3-4d.)	12-27-7 12-27-7	1-4-8 1-6-8 (8-10d.)	100	♂	♂♂	♀♀	♀♀♀	(♂)	1-16-8	12	29
3	3	1-13-8	8-10	5	15-16	sand	1-19-8 1-19-8	1-23-8 1-24-8 (4-5d.)	1-23-8 1-23-8	1-30-8 2-2-8 (7-10d.)	100	♂	♂	♀♀♀	♀♀♀	(♂)	2-12-8	13	30
3	4	1-16-8	10-15	5	15-16	sand	1-22-8 1-22-8	1-25-8 1-26-8 (3-4d.)	1-25-8 1-25-8	2-2-8 2-4-8 (8-10d.)	99	♂♂	♂	♀♀	♀♀♀	(♂)	2-13-8	14	31
4	3	2-12-8	8-10	5	15-16	sand	2-18-8 2-18-8	2-20-8 2-22-8 (2-4d.)	2-20-8 2-20-8	3-5-8 3-7-8 (12-14d.)	98	♂	♂	♀♀♀	♀♀♀	(♂)	3-15-8	10	31
4	4	2-16-8	20-24	4	15-16	sand	2-21-8 2-21-8	2-22-8 2-23-8 (1-2d.)	2-22-8 2-22-8	3-0-8 3-2-8 (13d.)	100	♂	♂♂	♀♀	♀♀♀	(♂)	3-21-8	12	33
5	3	3-15-8	12-18	5	15-16	sand	3-21-8 3-21-8	3-24-8 3-25-8 (3-4d.)	3-24-8 3-24-8	4-4-8 4-7-8 (11-14d.)	98	♂	♂♂	♀♀	♀♀♀	(♂)	4-14-8	10	30
5	4	3-21-8	10-15	4	15-16	sand	3-26-8 3-26-8	3-28-8 3-30-8 (2-4d.)	3-28-8 3-28-8	4-5-8 4-8-8 (8-9d.)	99	♂	♂	♀♀♀	♀♀♀	(♂)	4-17-8	12	27
6	3	4-14-8	8-10	4	15-16	sand	4-19-8 4-19-8	4-20-8 4-22-8 (1-3d.)	4-20-8 4-20-8	4-28-8 4-30-8 (8-10d.)	100	♂	♂	♀♀♀	♀♀♀	(♂)	5-9-8	11	25
6	4	4-17-8	15-20	5	15-16	sand	4-23-8 4-23-8	4-26-8 4-27-8 (3-4d.)	4-26-8 4-26-8	5-2-8 5-5-8 (6-9d.)	97	♂	♂	♀♀♀	♀♀♀	(♂)	5-14-8	12	27

LEGEND

The number of eggs was estimated, as suggested by Murdoch and Smart (132), on the basis that a spherical mass of eggs 0.5 cm. in diameter will give rise to 150 maggots.

Dashes indicate that egg viability and egg-laying capacity were still satisfactory at the time the old colonies of flies were destroyed and replaced by new colonies.

The number of eggs represents the approximate number laid each week on the basis of three collections per week.

TABLE 4. SUMMARY OF EGG-LAYING CAPACITY AND EGG VIABILITY

Gen. No.	Cage No.	Oviposition Began Date	Approx. No. Eggs Laid per week 3 Collections	Hatching Time Hrs.	Viability Decreased Date	Egg-Laying Capacity Decreased Date	New Gen. Started Date	Days Since Oviposition Began
1	1	11-11-7	5400	8-10	---	---	11-29-7	18
1	2	11-10-7	5025	8-10	---	---	11-29-7	19
1	3	11-11-7	6000	18-24	11-30-7	---	12-2-7	21
1	4	11-12-7	5100	20-24	12-1 -7	12-1-7	12-4-7	22
2	1	12-10-7	5325	8-10	---	---	12-27-7	17
2	2	12-9-7	5550	10-12	---	---	12-26-7	17
2	3	12-14-7	4950	10-15	---	---	12-29-7	15
2	4	12-18-7	5925	12-18	---	---	1-4-8	17
3	1	1-5-8	5925	10-12	---	---	1-25-8	20
3	2	1-2-8	5325	10-12	---	---	1-26-8	24
3	3	1-13-8	4875	8-10	---	---	1-30-8	17
3	4	1-16-8	5475	10-15	---	---	2-2-8	17
4	1	2-12-8	5325	8-10	---	---	2-26-8	14
4	2	2-12-8	5475	8-10	---	---	2-26-8	14
4	3	2-12-8	4875	8-10	---	---	3-5-8	21
4	4	2-16-8	5925	20-24	3-8-8	---	3-9-8	21
5	1	3-6-8	6000	15-20	---	---	3-22-8	16
5	2	3-9-8	5025	10-12	---	---	3-24-8	15
5	3	3-15-8	5325	12-18	---	---	4-4-8	20
5	4	3-21-8	5475	10-15	---	---	4-5-8	15
6	1	3-30-8	6225	8-10	---	---	4-14-8	15
6	2	4-4-8	5925	10-12	---	---	4-20-8	16
6	3	4-14-8	5025	9-10	---	---	4-28-8	14
6	4	4-17-8	6375	15-20	---	---	5-2-8	15

receptacles a corrugated[2] paper carton in which a hole about 8 inches in diameter has been cut out from the top center. Above the hole is suspended an electric light bulb in a metal lamp shade and fixed so that the shade can be adjusted either up or down (Fig. 1). The temperature desired is obtained by adjusting the lamp at the correct distance above the carton. Ordinarily, a carton large enough to house two fly cages is used, but a larger carton may be used just as satisfactorily if desired. This will depend upon the number of fly cages used. One medium-sized carton will be all that is necessary for the larval receptacles. If large numbers of larval receptacles are used, the best method is to place them on a small wooden rack.

Still another method of incubation, perhaps the simplest, consists of placing in the room a small electric heater, and the temperature of the room controlled by a thermoregulator suspended just above the fly cages and larval receptacles. A very economical and efficient thermoregulator is described in the section on apparatus for brood rearing.

Retardation in development of all the stages in the life cycle, where culture is being carried on in the laboratory without incubation may be obviated by any of these methods. Experience has shown that during warmer days this procedure is unnecessary. These methods of incubation proved to be of value only during colder nights

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[2] Since air exists in the corrugations, a good insulator is afforded.

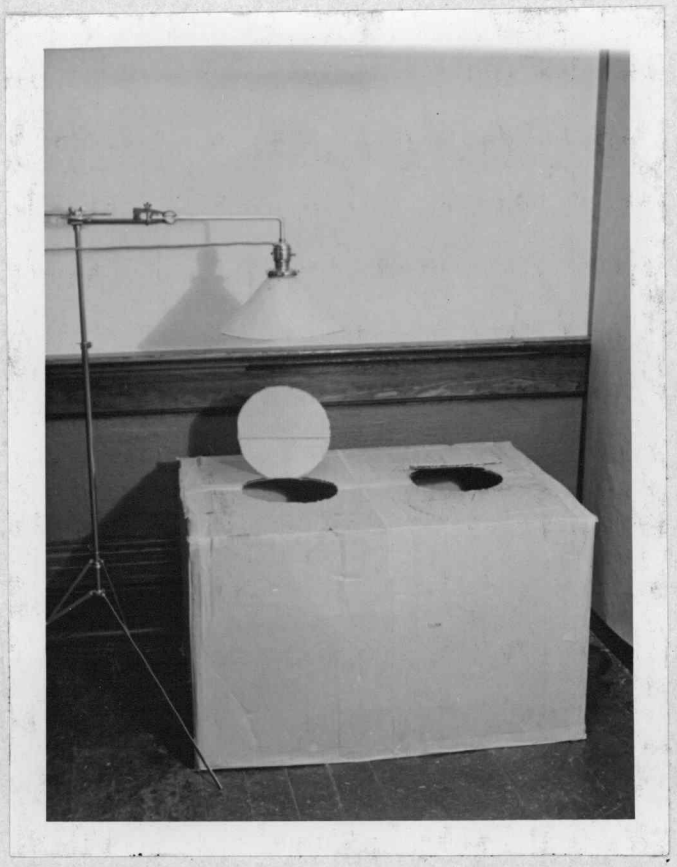

Fig. 1

or in cases where the temperature during the night would deviate much from that existent during the day. Location, climate, and the character of the laboratory are factors which determine the usefulness of any of these methods. Some laboratories may find the need for certain additional aids but these need not be elaborate or expensive. The author writes from the experiences in his own laboratory, and appreciates the fact that what applies to one laboratory may not necessarily apply to another laboratory. The technics described here are offered as suggestions rather than positive technics, and it is hoped that they may be of help to those who might be confronted with the problem of incubation.

APPARATUS FOR BROOD REARING

Fly Cages

Various types of fly cages have been devised for the housing of flies. These are either of screen or cloth, or a combination of both materials. Several types of satisfactory cages have been devised by the writer, and they will be described in greater detail.

Type 1 (Fig. 2). This fly cage, which is 16 inches square, consists of a wooden frame and floor. It is covered on three sides with ordinary wire screen and on top with transparent celluloid. The front of the cage is covered with fairly heavy cheesecloth in the center of which is cut a circular hole about 8 inches in diameter. Around the periphery of this hole is sewed a 14 inch sleeve of the

Fig. 2

same material. At the free end of the sleeve a 1/4 inch hem is made. Two strings are drawn through this hem so that the mouth of the sleeve can be closed easily. The sleeve facilitates entry into the cage and prevents the escape of flies when entrance is necessary. The top of the cage is made to open in door fashion by attaching two small hinges to the back of the cage. Two small hooks are attached to the front of the cage so that the top can be locked. This arrangement affords quick access to the inside of the cage when cleaning is desired, and facilitates rapid removal of dead flies when the colonies are destroyed. Another simple type of cover which allows convenient and rapid entry into the cage (Fig. 2) may be obtained by placing a wooden bar across the center of the top of the cage and fastening to the bar, by means of thumbtacks, a section of transparent celluloid. A border of adhesive tape is placed around this cover to increase its weights as well as to obviate cutting by the jagged edges. This arrangement has a spring effect and keeps the cover down tightly.

Type 2 (Fig. 3). This cage consists of ordinary wire screen and is shaped to fit into a 10 inch metal pan which serves as the floor of the cage. The cage measures 10 inches in diameter and 16 inches in height. The screen is held together by means of brass rivets, and all jagged edges of the screen are coated with liquid solder to prevent cutting. The top is fitted with a 14 inch cloth sleeve to facilitate entry into the cage. A 1/4 inch hem is made at each end of the sleeve, and two strings are drawn through each hem for fastening one end to the cage and for keeping the free end closed.

Fig. 3

The screen may be reinforced, if desired, by fastening it around a frame such as described in the construction of fly cage, Type 3. This type of cage is very economical, easily assembled, and extremely easy to clean.

Type 3 (Figs. 4 and 5). This cage consists of a circular frame of galvanized iron and wood, 10 inches in diameter and 16 inches in height, around which is placed a cloth cover containing a sleeve, described above, for entry into the cage. One-inch strips of wood and galvanized iron are used in making the frame, and these are fastened together by means of flat-headed bolts and nuts. The cloth cover is made to fit snugly around the cage and a 1 inch border is allowed to extend beyond the periphery of the top and bottom of the cage for fastening. To fasten the cover to the cage, all that need be done is to turn down over the edges of the galvanized iron rims the extending borders of the cover, and then fasten by paper clips. A convenient method of placing the cover on the cage consists of sewing a zipper on the cover. This facilitates greater ease in arrangement and removal of the cover. If a zipper is used, a sleeve is not necessary, as it was found that entrance into the cage could be made satisfactorily by partly opening the zipper. In the placement and removal of food, however, the opposite side of the cage is illuminated near the top by means of a desk lamp. This attracts the flies and obviates the danger of their escaping. The zipper should be arranged so that opening may be made from the bottom upward. The top and bottom

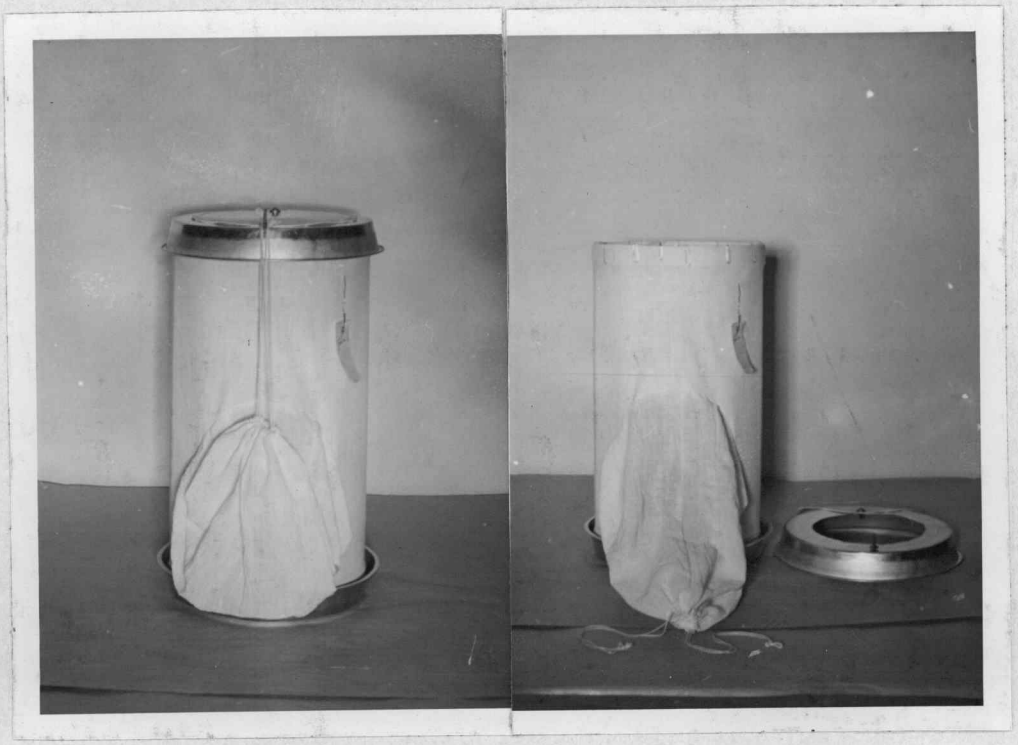

Fig. 4

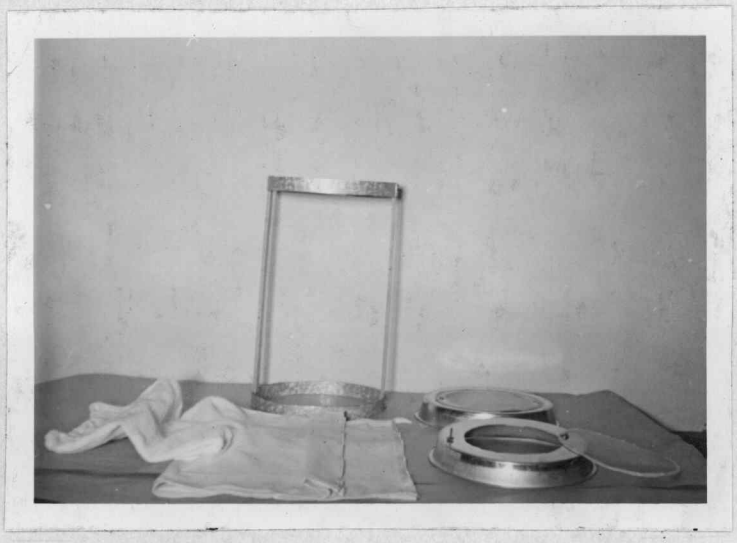

Fig. 5

of the cage consist of two 10 inch metal pans, each containing a circular opening about 8 inches in diameter and covered with a 9 inch transparent celluloid disk. The bottom disk is permanently fastened to the pan by two small flat-headed bolts and nuts, while the top disk is fastened to the pan with one small loosely-fastened bolt and nut so as to permit access into the cage during the transference of flies from soiled to clean cages. Transference is accomplished by inverting the clean cage over the soiled one and then illuminating the bottom of the inverted cage after the intervening disks have been moved outward. The flies, being positively phototropic, are quickly attracted to the illuminated surface. When the flies have migrated to the clean cage, the disks are moved back in place and the inverted cage is placed upright. The transparent disks also permit observation into the cage, a convenience especially helpful in the placement and removal of food dishes. This type of cage was devised in order to compare it with the screen type, since it has been pointed out by Simmons (172) and also by Murdoch and Smart (132) that screen is less desirable than cloth because flies break their wings on the screen by flying too strongly against it, and thus impair their normal functions. A record of injuries is included in the summary of fly mortality, Table 1, but no noteworthy differences were manifested in the cloth type of cage (Cage 1) as compared with the screen type of cage (Cages 2, 3, and 4).

Type 4 (Figs. 6 and 7). This cage is very similar to the one described by Simmons (172). It consists of a circular base and top of wood, 10 inches a diameter, and joined together by two galvanized iron strips measuring 16 inches in length by 2 inches in width, bent at their ends and fastened to the wooden disks by flat-headed bolts and nuts. The top has a circular opening 8 inches in diameter and is covered with a 9 inch disk of transparent celluloid or screen. The base contains a semi-circular opening 8 inches in diameter and on the under surface is thumb-tacked a section of transparent celluloid. The solid half of the base serves to hold food dishes, while the other half is used as an illuminating surface for the transference of flies from soiled to clean cages. Transference of flies is accomplished by the method described under fly cage, Type 3. The cover consists of cloth containing a sleeve for entry into the cage, and is fastened to the top and bottom by means of a cord which fits into a slot cut around the edges of the wooden disks.

Brood Larval Receptacle

The brood larval receptacle (Fig. 8) consists of a one-pound coffee can, a glass crystallizing dish (or similar container) for a feeding vessel, and some sand for the pupation of larvae. This device requires no renewal of parts and has proved very satisfactory as an all-round brood receptacle. To facilitate a free circulation of air through the receptacle, which is necessary in brood production, the greater portion of the cover of the can is

Fig. 6

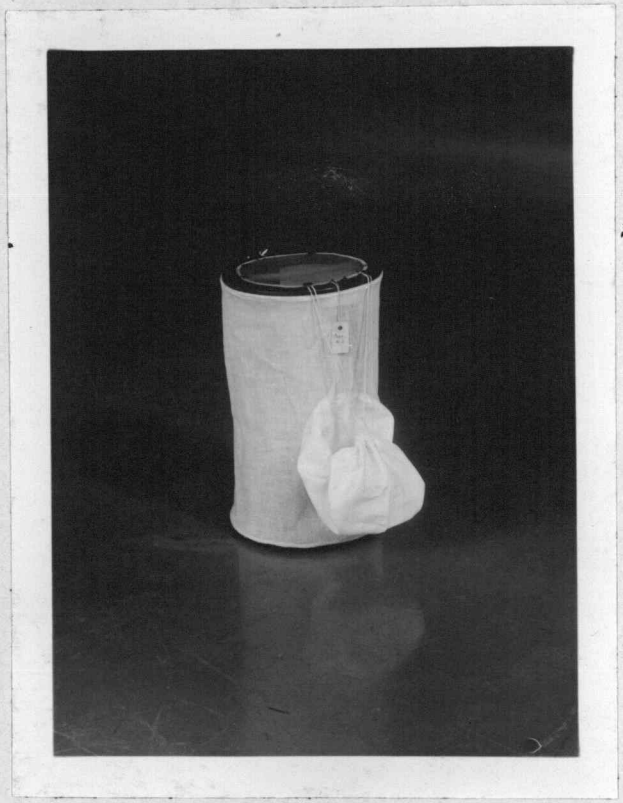

Fig. 7

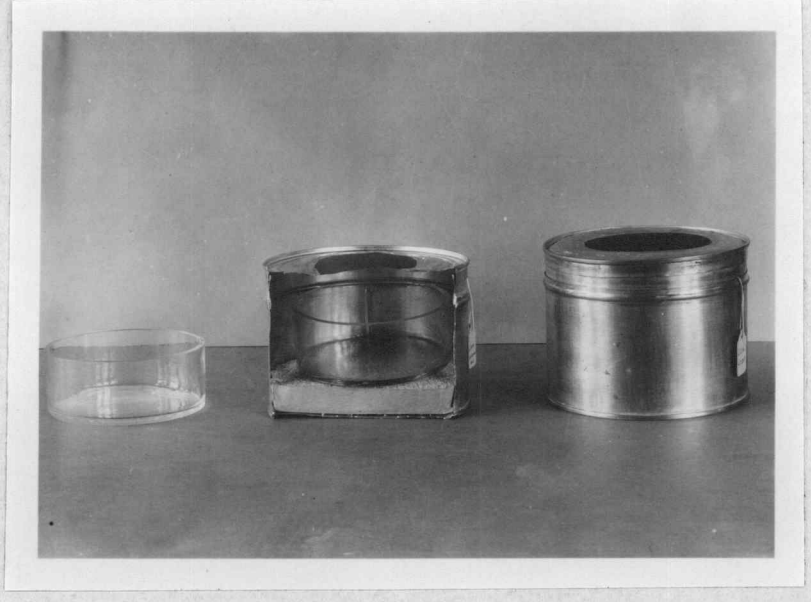

Fig. 8

cut out and replaced with a 60-mesh brass wire cloth[3] which is fastened down with liquid solder. The close fit of the cover over the top of the can prevents larvae from making their way between the can and the cover where they would be crushed when the can is opened, as is the case when jars with grooved metal covers are used.

Pupae Collector

By means of the pupae collector (Fig. 9) pupae can be separated rapidly from the sand in which they form (Fig. 10). The collector consists of wire and door screen and is made as follows: First, the wire is shaped to simulate a spoon, the two ends being fastened together with a small piece of wire and covered with liquid solder to make the joint firm. Next, the screen is cut so that a 1/2 inch border extends beyond the periphery of the spoon. Then, the 1/2 inch border of the screen is folded over the periphery of the spoon, trimmed down, and coated with liquid solder, the solder serving not only to fasten the screen to the wire frame but also to prevent cutting by the jagged edges of the screen.

Egg Collector

Because it is not always possible to collect eggs soon after they are deposited, many of them are desiccated as a result of being

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[3] A finely perforated section of transparent celluloid may also be used.

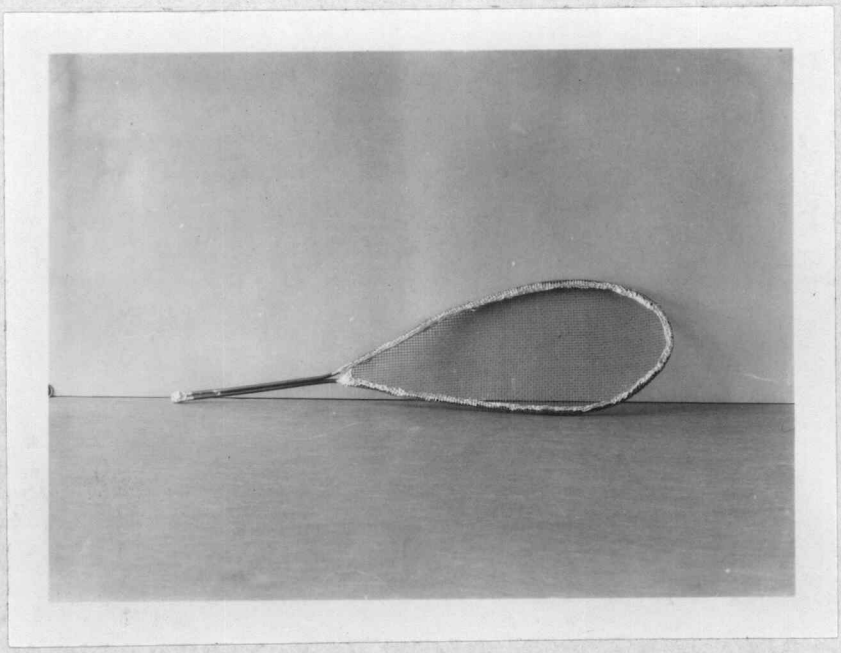

Fig. 9

Fig. 10

exposed too long to the laboratory air, even in cases where the humidity is relatively high. The meat used to collect eggs becomes dry and leathery when exposed too long to the air and as a result, the feeding surface becomes extremely poor and the flies are less readily attracted to it. The device shown in Fig. 11 was found to besatisfactory in keeping both the meat and the eggs in good condition for longer periods of time. This consists of a glass stender dish, measuring 100 by 100 mm., which is used as a water receptacle. In it, acting as a tray, is hung a metal jar cover which is slightly smaller than the diameter of the stender dish. Two pliable metal strips are screwed to the border of the jar cover and adjusted so that the cover can be placed at any desired height above the water. The stender dish is filled with water to a depth of two inches. A small absorbent cloth skirt is lapped over the tray which is then suspended in the stender dish about 1 inch above the surface of the water, with the cloth skirt hanging in the water. The skirt serves to draw up water by capillarity, to increase the surface of evaporation, and to fill the space between the cover and the wall of the receptacle and so prevent flies from making their way to the water and drowning. On top of the cloth are placed two sheets of filter paper. On the filter paper is placed a small pat of ground, lean beef in which rather deep, obliquely directed pockets are made by pressing a finger into its lateral and upper surfaces. The meat is kept moist by the filter paper which draws up water from the cloth skirt. The flies appear to be quite fond of these little pockets and

Fig. 11

deposit their eggs in them. The pockets also serve to keep the eggs from being exposed too much to the laboratory air, and make it possible to leave eggs for longer periods of time without the danger of being desiccated.

Another method of preventing too much loss of water from the tissues of the eggs, which was found quite satisfactory, consists of arranging the meat so as to allow the flies to get in under it to lay their eggs, which they invariably do. This is easily accomplished by using thin sections of meat. In this manner, some warmth is afforded, as well as a little moisture which results from a "sweating" action of the meat around the eggs. During very dry periods a strip of cotton saturated with water and placed loosely over the meat covering the eggs will be found helpful in preventing excessive drying.

Brood Incubator

The brood incubator (Fig. 12) may be made from an old box or cabinet. The one described here consists of a reconditioned pastry cabinet with a wooden back, glass sides, and a glass door; and measures approximately 21 inches wide, 21 inches deep, and 67 inches high. It contains three shelves, each having a 3/4 inch border cut away on all sides and an extension at each corner which rests upon a support. This shape is used to facilitate a good circulation of air. On the undersurface of the bottom shelf is attached a sheet of insulating aluminum foil to prevent warping of the shelf by the heating unit

Fig. 12

below. The heating unit consists of two porcelain electric sockets, each containing a 125-watt electric light bulb. Humidity within the incubator is supplied by a shallow pan of water placed opposite the heating unit. If desired, humidity may easily be increased by immersing a heavy wad of cotton, cloth, sponge, etc., in the pan of water, or by suspending several strips of heavy absorbent cloth in the pan. Ventilation is maintained by several 3/8 inch holes bored in the lower right-hand and upper left-hand sides of the incubator. Air enters at the bottom and leaves at the top. The circulation can easily be regulated by plugging some of the holes with corks. This is done from the inside of the incubator.

Both the humidifier tray and heating unit are located on the floor of the incubator, the latter on the side where the holes for ventilation have been bored. At the top of the incubator a small section, with wooden sides and back, serves to hold larval receptacles. The fly cages are placed on the shelves below.

A convenient arrangement for bringing water into the humidifier tray is to invert a quart jar on a ring stand attached to the upper outer side of the incubator. The bottom of the jar, which now serves as the top, is cut off, so that water can be poured in and is kept covered with a metal lid. Over the mouth of the jar is screwed a tin cover in which a hole is cut to receive a cork fitted with a glass tube. Attached to the glass tube is a rubber tubing at the end of which is a double right angle bend glass tube. A cork, fitted into a hole in the lower outer side of the incubator, holds this right angle tube in place. The water is kept from flowing out by a pinchcock.

Thermoregulator

For regulating the temperature the thermoregulator described by M. C. W. (120) was found very satisfactory.[4] This device (Figs. 13 and 14) consists of a 3/4 inch ether-wafer unit,[5] originally intended for a poultry incubator. The wafer unit, which has a short threaded stud projecting from the center of the bottom surface, is fastened to the supporting bar (a) with a small nut (b). The wafer unit also has a hollow-end stud projecting from the center of the top surface, which receives the end of the adjusting rod (c). Two metal contact strips are adjusted on the base piece (d) so that one (e) is fastened to the adjusting rod by means of a small nut (f) which serves to support the adjusting rod (c). The other strip (g) is adjusted so that it makes contact with strip "e" at the proper point.[6] The adjusting rod is threaded for some distance so that the temperature may be increased or decreased. When the wafer unit is warmed, the ether vapor inside expands and forces the hollow-end stud up against the end of the rod (c), causing the movable contact strip (e) to bend upward and break the contact. This turns off the heating unit. Loss of heat by circulation and diffusion through the sides of the incubator

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[4]The construction of the thermoregulator described here was modified somewhat by the writer.

[5]These come in different sizes and range in price from 35 to 60 cents. The size used will depend upon the dimensions of the incubator.

[6]Excellent contact strips suggested by M.C.W. are those which can be obtained by tearing apart an old telephone jack, which can be secured at a radio repair store or where miscellaneous radio and telephone equipment is available. These strips are already provided with contacts that produce a minimum of sparking.

Fig. 13

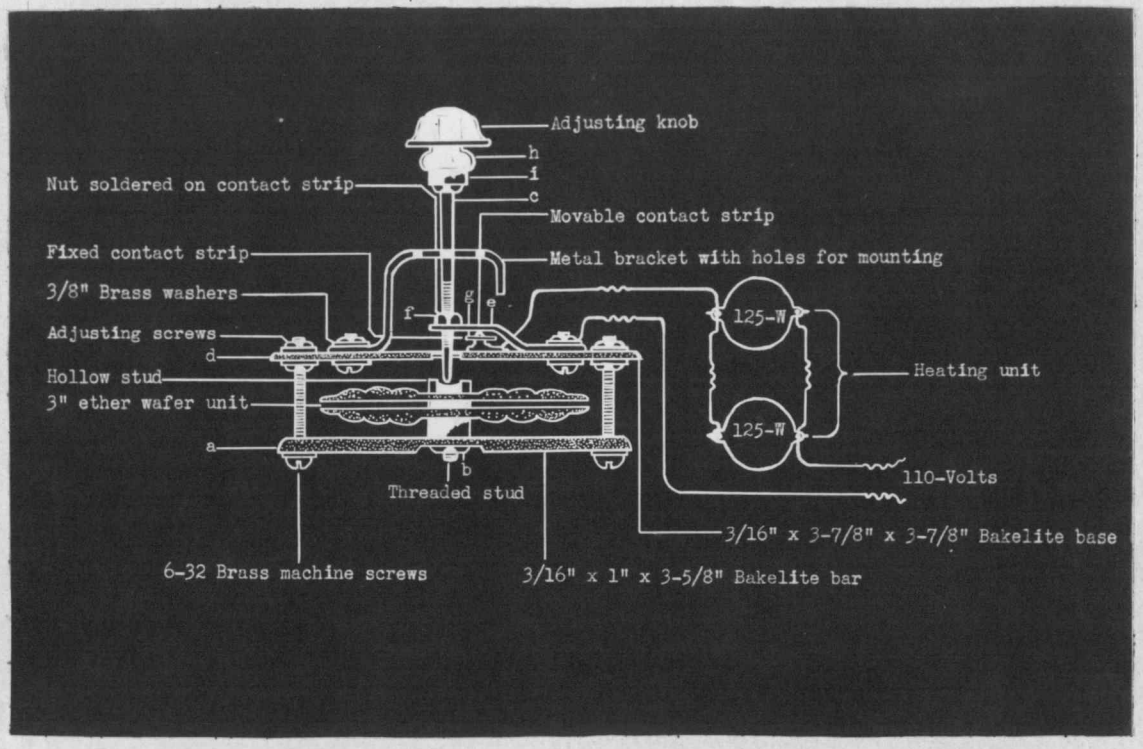
Adjusting knob
h
i
c
Movable contact strip
Nut soldered on contact strip
Fixed contact strip
Metal bracket with holes for mounting
3/8" Brass washers
g
f
e
125-W
Adjusting screws
d
Heating unit
Hollow stud
3" ether wafer unit
125-W
a
b
110-Volts
Threaded stud
3/16" x 3-7/8" x 3-7/8" Bakelite base
6-32 Brass machine screws
3/16" x 1" x 3-5/8" Bakelite bar

Fig. 14

causes the temperature to drop, the wafer unit to contract, and the contact points to come together again. The lamps in the heating unit light; the disk expands and again breaks the circuit when the predetermined temperature has been reached. If it is desired to make the thermoregulator break the circuit at a higher temperature, the adjusting rod is turned in a counter-clockwise direction. If a lower temperature is desired, the rod is turned in a clockwise direction. A helpful suggestion will not be out of place at this point. In some cases it was difficult to get the contact strips to touch exactly. For this reason, sensitivity was not very great. After some experimentation, however, it was found that by increasing the weight of the adjusting rod the strips could be made to touch each other and thus greater sensitivity could be effected. This was accomplished by fastening two lead washers (h and i) under the adjusting knob. The washers were made by pouring melted lead into a mold of the desired size. Holes were made in the washers by placing a greased metal rod in the center of the mold before pouring in the lead. With the aid of this adjustment the temperature was made to vary less than 0.4 of 1° C.

METHOD OF BROOD REARING

The pupal stage will be found a very convenient starting point in establishing the fly colonies. Pupae are easily handled and large numbers may be shipped at a nominal cost. Laboratories

culturing surgical maggots are very willing to supply a sufficient number of pupae for establishing fly colonies.

Approximately 150 pupae are placed into one half of a petri dish and covered with sand. The sand is used to prevent the desiccation of pupae. It was found that unprotected pupae left exposed in the laboratory frequently resulted in a marked mortality due to desiccation. By the use of sand an average hatching of 98 per cent was obtained. The pupae should not be buried too deeply in the sand, as then the emerging flies find it difficult to make their way to the surface. This finding greatly simplified the matter of pupae handling in the laboratory.

When the pupae dishes have been prepared one dish is placed into each fly cage. As soon as the emergence of flies is noted, food is placed into the cages. The food simply consists of plain cane sugar in brick or domino form, and water, and is kept in the cages constantly. This food was recommended by Haub and Miller (78), who worked with blowflies of the species Lucilia sericata and Phormia regina, and showed experimentally that this food was all that was necessary for the carbohydrate requirement of the flies. The sugar in the brick form substituted all other commonly used foods such as honey, syrups, honey-water-yeast mixtures, vegetables, fruits, etc., and has simplified greatly the matter of feeding as well as cage cleaning. As a source of protein, plain lean beef was found the most satisfactory for the flies as well as for the maggots. These foods

were used throughout the entire course of this investigation and have proved very satisfactory. Water is supplied by means of a fountain (Fig. 15) also described by Haub and Miller (78). This consists of a small beaker of water inverted upon one or two sheets of filter paper in a petri dish. Water is emitted at the beaker lip and soaks out into the paper. When the water is consumed or evaporated, air enters at the lip and forces more water out. This always keeps the paper wet, but no excessive quantity is present at any time in which the flies may drown.

Small pieces of meat, approximately 3 inches square are placed into the cages for the flies to feed upon. The meat is placed into the cages as soon as the flies begin to emerge and is added daily or every other day until oviposition begins. It is added subsequently only when it is desired to collect eggs. If the meat is turned over and moistened occasionally, it may be used for more than one day. The meat may be kept moist by placing it upon a wet cotton pad in a petri dish. To afford a better feeding surface the meat is partially chopped or ground. If drying is excessive, the egg collector described above may be used advantageously. During egg collection the food dishes are removed and larger pieces of meat than during feeding should be used so as to afford sufficient egg laying surface. Flies will not lay eggs too near areas already containing eggs. The flies continue to lay eggs well for about 3 to 4 weeks, after which time egg production and viability of eggs decrease. When this condition occurs the flies are destroyed and

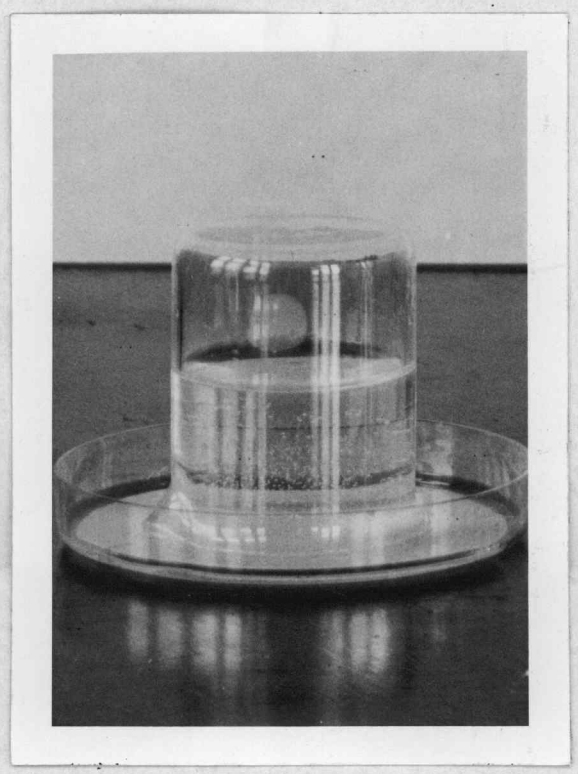

Fig. 15

replaced by new colonies.

Larvae are reared in the brood receptacle described above. In preparing the receptacle for use, the meat upon which the eggs have been deposited is placed in the feeding dish which is well provided with food, and the feeding dish is placed into the can which is then covered. The eggs should be protected against possible desiccation, and the food should be moist. Too many eggs should not be placed in one receptacle, since, as was pointed out above, overcrowding results in premature migration of the maggots from their food, giving rise to small flies with low egg-laying capacity. For a receptacle of this size, approximately 500 to 600 eggs is a satisfactory number. The number of eggs may be determined roughly as suggested by Murdoch and Smart (132) on the basis that a spherical mass of eggs 0.5 cm. in diameter will give rise to 150 maggots, or by the more exact weight method used by Robinson and Simmons (162) on the basis that on the average 9.9 eggs weigh one milligram. One soon learns by experience how to judge numbers by the size of the egg masses, and measuring or weighing becomes unnecessary.

As soon as the larvae appear full grown (approximately 15 mm. in length) about an inch of sand is added to the can for pupation. When nearly all the larvae have migrated from the feeding dish, the dish and the remaining food are removed, and the larvae are left to pupate. Sand is almost invariably added to the larvel receptacles when they are being prepared for use, but this practice was not found desirable because larvae often leave their food before

they are mature; and if sand is present they will enter it, pupate prematurely, and develop as a result into small flies with low egg-laying capacity. When sand was not added to the receptacles until the larvae appeared full grown, it was observed that many of the larvae that left their food prematurely returned to it, resumed feeding, and reached maturity normally.

When all of the larvae have assumed the pupal form, the pupae are placed into petri dishes, and covered with sand as described above. The dishes are then placed into the fly cages and left there until the pupae have hatched.

Elimination of Odors

In the production of brood larvae disagreeable odors develop, and some care should be exercised to prevent them from becoming noticeable in the laboratory. An exhaust draft attached to a special larval incubator has been the method almost universally recommended for ridding the laboratory of odors, but such a procedure is rather expensive. The need for a more simple method of relieving the laboratory of such odors presented itself, and after some experimentation, several methods were found satisfactory. These are quite simple and inexpensive.

In brood rearing odors are most marked at the beginning of the feeding period and usually persist for about two days. After this time, however, their initial potency gradually diminishes and is overcome by the odor of ammonia which results from the liquefaction

of the meat by the larvae. For this reason it is best to use a piece of meat large enough to carry through the feeding period and to use smaller lots of maggots. It was found that frequent additions of meat tended to renew the odors because digestion and decomposition had to be started anew. During the first two days the larval receptacles are placed in a small portable incubator, and the incubator is then placed in a ventilating hood. If a hood is not available the incubator can be easily transferred to some unoccupied room in the building, and left there until the feeding period has been completed, or until the odors are no longer noticeable.

If it is desired to ventilate the room, a quite satisfactory method is to open a few inches from the top a window at one end of the room, and a few inches from the bottom a window at the other end of the room. Some laboratories are already equipped with ventilation fans of one type or another, and if such are available, they would be satisfactory.

There are also now available various types of deodorizing machines, and many laboratories are using them. Most of these machines are quite satisfactory, but they are, as yet, rather expensive.

The methods used in ridding the laboratory of odors were discussed in hope that they would lead to helpful suggestions. It is felt that no definite method can be recommended, since the

character of the laboratory and the facilities available will best decide this matter.

Since it is not always possible to attend immediately to newly hatched flies, it was desired to find out how long such flies could be left without food and water before mortality occurred. It was found that flies could be kept alive after emergence from the pupal stage in the incubator for about 4 days, without food and water before mortality occurred; and in the laboratory for about 5 days.

CULTURE OF STERILE MAGGOTS

The danger of introducing pathogenic organisms into the wounds with non-sterile maggots and the importance of employing sterile larvae in the practice of maggot therapy have already been brought to view under the discussion of Baer's early research. Aseptic bacteriologic technic is of the utmost importance throughout the entire process.

Since Baer (11) described his technic of sterilization, many other methods have been described in the literature, and most of these have proved satisfactory. The method described here is the one devised by Simmons (173) of the U. S. Bureau of Entomology. His method is simple, rapid, economical, and efficient, and eliminates

the necessity of retarding the growth of eggs by cold storage[7] for sterilization the next day, as is the general practice. Eggs may be laid, collected, and sterilized within one day. Fresh eggs (up to two hours of age) are recommended for sterilization.

Before sterilization is begun, the eggs, which are almost invariably laid in masses, are carefully separated so that the entire surface of each one is covered by the disinfectant. Failure to separate the eggs completely may result in incomplete sterilization. Separation is accomplished by merely placing the eggs on a wet cloth[8] which is moistened by being placed on a wet cotton pad in a covered petri dish (Fig. 16). Eggs which are collected in large clumps are best separated by placing them on one-half of the cloth and turning the other half over them. In a few minutes the eggs can be separated easily by spreading them thinly on the cloth with a spatula. The longer the eggs are left on the cloth, the more easily they can be separated. This process softens the mucoid substance around the eggs, and thousands of them may be separated without injury.

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[7] Robinson and Simmons (161) in their study "Effects of low temperature retardation in the culture of sterile maggots for surgical use," make the following conclusion: "Low temperature retardation of the various stages in the life cycle of the blowfly, although a convenience in cultural technic, causes such a high mortality that its use is considerably limited. It appears to be best adapted to short storage periods or when the convenience of continued storage is sufficient to counterbalance the losses."

[8] The use of black cloth is recommended. This aids in the detection of minute clumps of eggs.

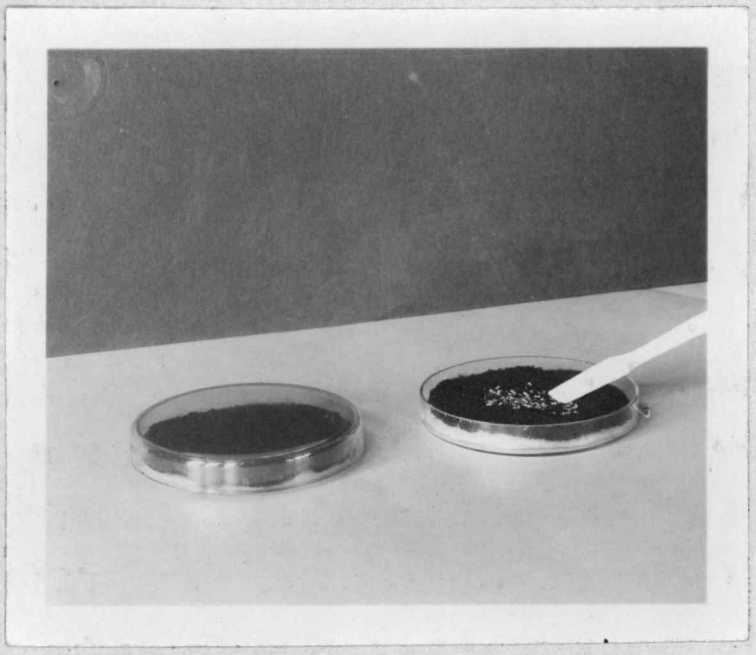

Fig. 16

The eggs are next transferred to the disinfectant in a test tube by means of a spatula, and then stirred gently with a wooden applicator (Fig. 17). The disinfectant consists of a 5 per cent solution of formalin containing 1 per cent of sodium hydroxide, and is made up fresh just before use. The sodium hydroxide prevents the agglutination of the eggs in the disinfectant, a tendency which exists in eggs separated by the wet-cloth method. The eggs are kept in the disinfectant for five minutes, after which time most of the solution is decanted and the remaining solution containing the eggs is poured on to a small piece of gauze in a Gooch crucible supported by a wide-mouthed specimen bottle. About 50 to 100 c.c. of sterile water is then poured slowly over the eggs to remove the disinfectant. A glass cap covering the crucible and neck of the bottle serves to maintain the sterility of the eggs until ready for use. Another method of washing the eggs, which the writer has found satisfactory, consists of pouring the eggs on to a piece of gauze in a small filter funnel. The tip of the funnel is fitted with a small section of rubber tubing containing a glass pipette. The tubing is clamped with a pinch-cock for regulating the flow of water. The top of the funnel is kept covered with a crucible cover.

After sterilization and washing, the sections of gauze containing the eggs are transferred, by means of a sterile forceps, directly to the feeding receptacles containing sterile food. The gauze may be removed from the receptacles after the eggs have hatched, or left undisturbed until the maggots are ready for implantation.

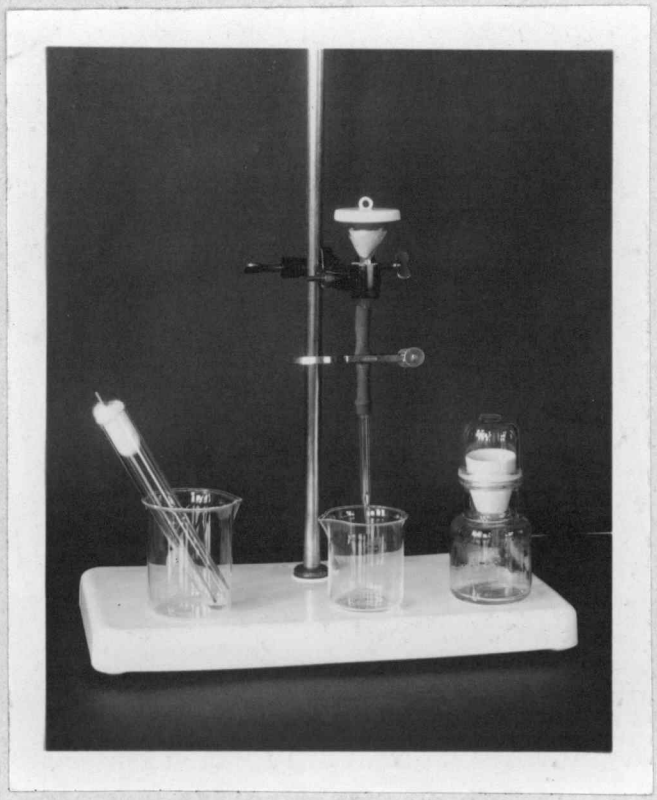

Fig. 17

Various types of containers have been used in the culture of sterile maggots. The writer has found 4 ounce packers jars very satisfactory (Fig. 18).

Aseptic rearing of maggots should be carried on in small numbers, for if contamination is detected after sterilization the loss is correspondingly small. About 500 to 600 maggots to each container will be found a satisfactory number.

Food for Sterile Maggots

There are two types of food available for the culture of sterile maggots. One type enables rapid growth of the maggots, but necessitates cold storage for keeping the maggots retarded in growth during the sterility tests. The limitations of cold storage for the retardation of growth have been indicated above. Several formulas of this type of food are in the literature.

The other type of food, devised by Simmons (174), permits only a slow rate of growth up to the time the maggots are placed into the wound, but this does not interfere with their feeding activities and development after implantation. This food consists of one part of fresh evaporated milk to from five to seven parts of water, to which is added 1.5 per cent plain agar, and is prepared as follows:

First the milk and water are mixed; then the agar is added, and the mixture is cooked in a double boiler for about 25 minutes;

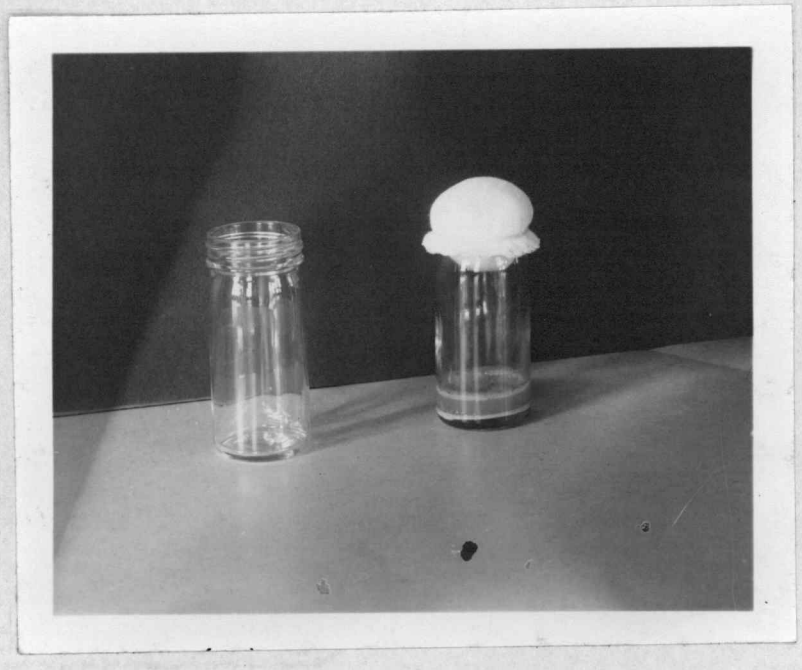

Fig. 18

while still hot, about 15 c.c. of the food are poured into each feeding receptacle, which has been previously plugged with gauze-covered cotton and sterilized. Concerning this food, Simmons (174) states:

> The proportion of milk and water is sufficiently nutritious to sustain the larvae without allowing material growth and development. Some latitude in concentration is tolerated, however, any ratio of from 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.

After the food is added, the receptacles are replugged, autoclaved for 30 minutes at 15 pounds' pressure, and stored in the refrigerator or in the laboratory until used. The maggots may be kept at either room or incubator temperature. One is therefore able to hold surplus maggots in reserve for a few days without chilling and consequent mortality. This also permits the technician to interrupt work for short periods when other duties become more pressing. The surgeon is frequently unable to use the maggots promptly after being received, and for this reason it is both convenient and economical to be able to hold them over until needed. Simmons (174) has shown that the rate of growth and development of the maggots could be retarded for as long as 8 or 9 days without serious injury. The cost of culture is further reduced since there is no need for refrigeration. Another advantage

of this retarding food is that sterile maggots may be shipped over long distances without ice packing. This has not only reduced the cost of mailing considerably, but it has also greatly simplified the problem of shipping in general.

Test for Sterility of Maggots

To make certain that each lot of maggots is free of contamination, tests for sterility are made about 48 hours after the eggs have hatched. Both aerobic and anaerobic tests are made from some of the partly liquefied food upon which the maggots are feeding. This method of testing is simple as well as reliable. After 48 hours the cultures are observed, and if found to be negative, the maggots are ready for us.[9] By this time the maggots will have grown to about 6 mm. in length, a satisfactory size for clinical use. Larger maggots are less desirable, since their feeding period in the wound is too short to do much good.

Collecting Maggots From Cultures

When the maggots are ready for implantation, sterile water or normal saline solution is poured into the culture receptacles so as to suspend them, and the maggots are then collected with a sterile

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[9]As an additional precaution, some laboratories incubate negative cultures several days longer, after the maggots have been released.

spoon or tongue depressor and transferred to sterile bottles. They are then washed once or twice to separate them from the food. Cold water is used to subdue the maggots so that they will not escape. After being washed, the maggots are placed into the wound. Another method of collecting the maggots consists of suspending them in water and then straining them through a fine-mesh wire strainer. They are then washed, strained again, and placed into the wound. Maggots are frequently transferred to wounds directly from the feeding receptacles. This is done by suspending the maggots in water and then collecting them with small pieces of sterile gauze as the solution is poured from the containers. As they are collected, the maggots are dropped into the wound. Small amounts of food material frequently adhere to the maggots, but no harmful effects in the wounds from this have ever been reported to the writer's knowledge.

TREATING INFECTIONS WITH MAGGOTS

Before maggot therapy is instituted, the infected area is opened wide so as to thoroughly expose it to the maggots. Unless this is done the treatment will be of little value. Opening the wound in this manner establishes adequate drainage as well as provides a favorable environment for the maggots, as they feed near the surface or in shallow openings and will not penetrate deeply into a sinus. The character of the operation and the manner of treatment

will depend on the type of infection. Minor infections, as well as certain other types, need not be treated surgically.

After the operation, the wound is packed with sterile vaseline gauze and left there for 24 hours or longer until all the bleeding has stopped, as maggots are repelled by blood and do not function well in its presence.

The packing is next removed and the wound is cleansed with normal saline solution. From 200 to 1,000 maggots, depending upon the extent and condition of the infection, are then placed into the wound and the wound is covered with a wire screen, or with gauze, which is tightly fastened down to prevent the escape of the maggots.

Too many maggots should not be placed into the wound as overcrowding causes them to want to escape; this may result in pain and considerable annoyance to the patient.

Various types of retainers or cages have been devised for confining the maggots to the wound. Child and Roberts (34) have described one of the more satisfactory cages (Figs. 19, 20, 21, and 22).

> A pattern of the wound is cut from bandage gauze. A cage.....is prepared by cutting a piece of 80 mesh brass screening cloth to the pattern made from the bandage gauze. The edges of this screen are inserted between single strips of sponge rubber split longitudinally. The opposing surfaces of the sponge rubber are first coated with rubber cement and then sewed together with the screen margin between. The screen is sterilized in an autoclave and is then ready for use.....Strips of adhesive plaster, one inch wide, are placed on the skin all about the wound and flush with the wound edges. This is to protect the skin and prevent dermatitis. "Duo" adhesive is then painted on the adhesive plaster. This "Duo" is waterproof and more securely holds the cage in place and prevents the

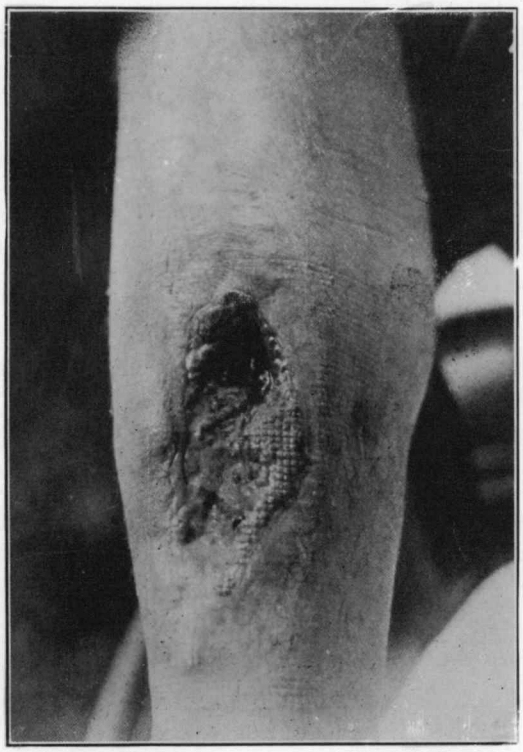

Fig. 19

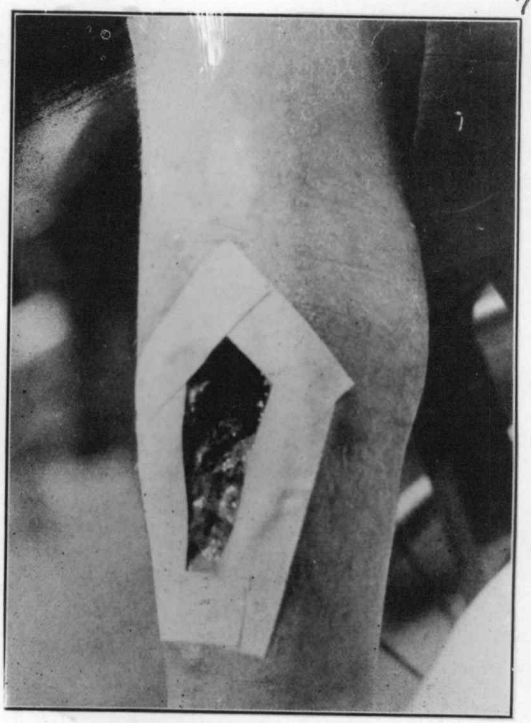

Fig. 20

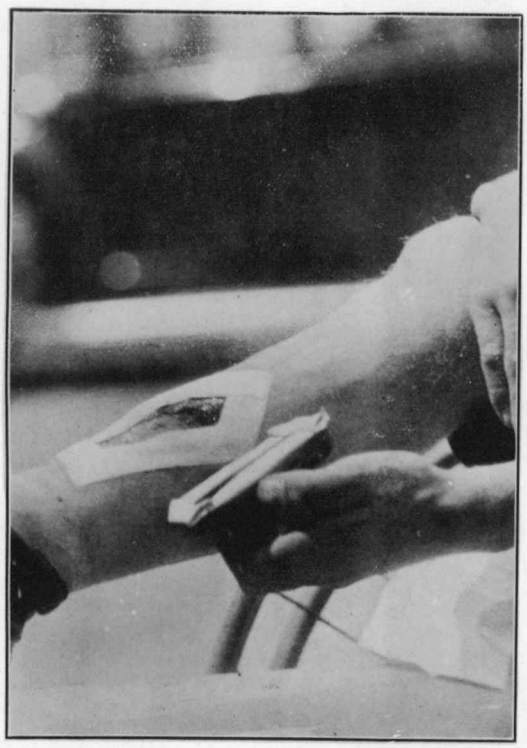

Fig. 21

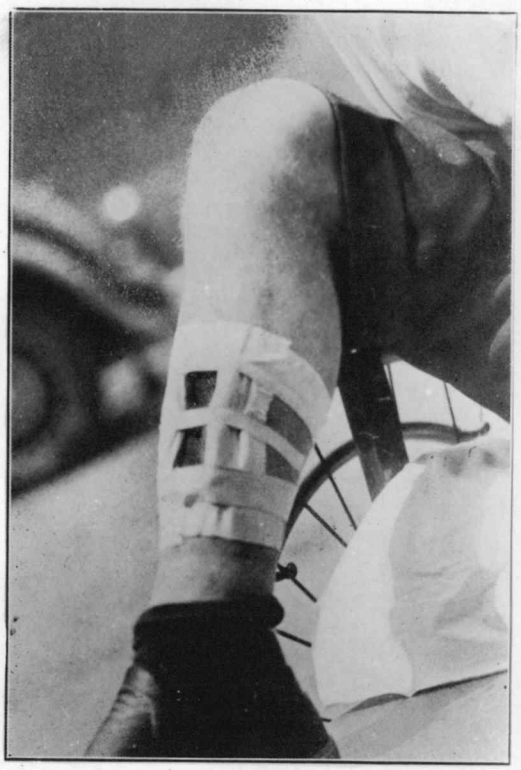

Fig. 22

> escape of maggots. Next, adhesive plaster the length of the cage is folded, adhesive surface out, and attached to the sponge rubber of the cage on the side which is to be against the wound.....The cage is secured in place by wide strips of adhesive extending from the sponge rubber to the skin, and then by narrow strips of adhesive placed at intervals across the cage.

A much more simple and convenient cage is described by Buchman and Blair (27). The edges of the wound are coated with liquid "Duo" adhesive and a section of fine muslin is simply placed over the wound.

Jewett (92) in his work has found Unna's paste very satisfactory for fastening the cage to the wound. The formula of this paste is as follows:

Zinc oxide.....	2-1/2 parts
Gelatin.....	6-1/4 parts
Glycerine.....	19 parts
Water.....	19 parts

Maddock and Jensen (109) have used quite a different idea for retaining the maggots in the wound. They describe their method as follows:

> Enough maggots are implanted to cover comfortably the surface of the wound,.....and then the wound is packed lightly with gauze. The capillary attraction of the gauze ensures continuous drainage and avoids pooling of secretions of pus. The entire wound is then covered with fluffed gauze held in place with bandages. Cellucotton pads are finally placed over the dressing in order to absorb the excessive wound drainage.

In some cases the ordinary cage can not be used; therefore, special types are required. Carbuncle of the neck is an example of

such a condition. The cage recommended by Ferguson and McLaughlin (49) (Figs. 23 and 24) has proved quite satisfactory for this type of infection, as well as for others where the surface of the wound is irregular or otherwise unsuited to use by the ordinary cage. They describe this cage as follows:

> Gauze containing maggots is introduced into cavity of carbuncle twenty-four to forty-eight hours after central excision and radial incision. Gauze compresses are applied and covered with crumpled wax paper. Dressing is secured with bandage and adhesive.

After the cage is applied, an electric light is frequently placed over the wound to drive the maggots in deeper, since they are repelled by light and seek the dark. If conditions within the wound are favorable, however, this is unnecessary. Some patients have reported a comforting sensation from the heat and the lamp is probably used more for this reason than for any other. Some believe that warmth is of value because it makes the maggots work better.

A considerable amount of serous liquid is secreted by the wound during maggot treatment, and means must be taken to keep the wounds well drained. If excessive secretion is permitted to accumulate, the maggots cease feeding and try to escape, and those that cannot get away are usually drowned in the wound. This retards healing and increases the cost of treatment.

The efficiency of the action of maggots in wounds depends greatly on how well sinuses have been opened up surgically and how carefully these tracts have been kept free from accumulating waste

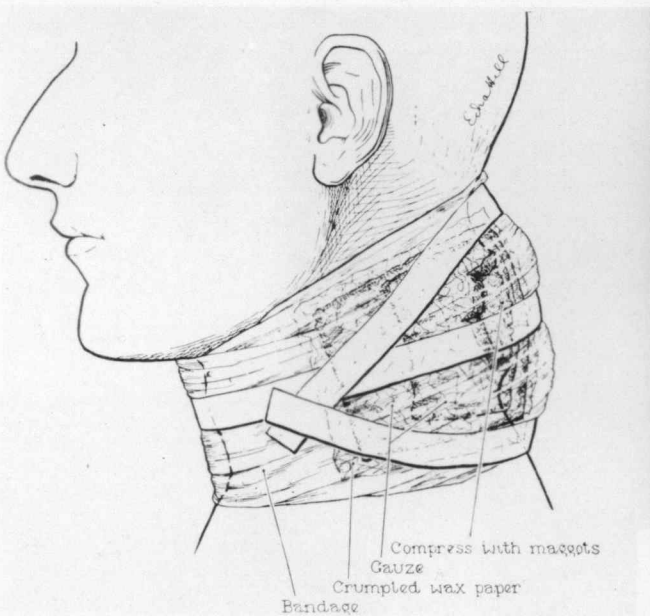

Fig. 23

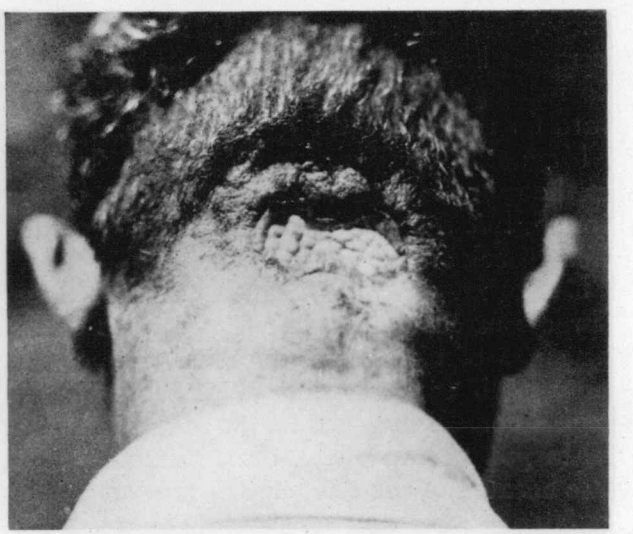

Fig. 24

products and other secretions. The wounds must be kept wide open so that the progress of healing may take place from the bottom upward. This is accomplished by various mechanical appliances called self-retaining retractors. These are usually of metal, and are readjustable so as to meet the different dimensions of the wounds.

The maggots feed upon the necrotic and purulent matter within the wound. In 3 to 5 days they reach their full growth and stop feeding. At this stage, no longer being useful, they are washed out of the wound with sterile normal saline solution. The patient is then allowed to rest for about 24 hours. At the end of that time, a new lot of maggots is introduced.

The length of time that maggots feed in the wound depends partly upon their size when they are implanted. The feeding period at the beginning of the treatment is longer than that toward the end of the treatment because maggots do not feed upon viable tissue and as the wound is cleaned up less food is available for them. When all the necrotic and decaying tissue has been devoured, the maggots die from insufficient nourishment.

The number of maggot implantations necessary depends largely upon the individual patient and the character of his wounds. The implantations range from 1 to 25, and sometimes more. For example, in children the healing period for chronic osteomyelitis of the long bones is about six weeks. In adults this period is at least a third longer.

CASE REPORTS

Thousands of results of cases treated with maggot therapy have been reported. A number of typical results are presented here.

Case 1 - J. L., age 15, female. Onset of acute osteomyelitis July, 1929. Admitted to St. Charles Hospital for Crippled Children August 22, 1930. Diagnosis, chronic osteomyelitis. Involvement right femur. Osteotomy and Orr treatment instituted September 10, 1930.

First implantation of maggots December 9, 1930. Last implantation May 15, 1931. One month later scar was covered with epithelium, which was smooth and without indentations. Total implantations, eighteen.[10]

Case 2 - R. H., aged seven. Recurrent osteomyelitis, right tibia. Partial sequestrectomy and wide drainage. Extensive sequestrum, involving practically the entire tibia, could not be completely exposed. Ten days after operation, 1,000 maggots introduced for five days. Three such operations followed by applications of maggots in a five month period. The wound healed nicely by granulation after the last operation.

The maggots had helped to demarcate and remove the dead bone, and were borne without complaint by this child of seven years.[11]

Case 3 - C. O., age twenty-five. Chronic osteomyelitis right ilium. A draining sinus had persisted for seven months. Incision and

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[10]Case 1. (Reported by Child and Roberts (34) as Case 1.).

[11]Case 2. (Reported by Ferguson and McLaughlin (49) as Case 2.).

wide open drainage of the tract. Three days later 500 maggots introduced for seven days. Wound much cleaner and granulating from bottom.

The maggots helped speed healing and clean up the detritus.[12]

Case 4 - W. C., aged fifty. Extensive carbuncle involving the entire nape of the neck and lower scalp. Central necrotic area excised and radial incisions made and undermined to form flaps. Wound packed with gauze in a shell of paraffin gauze for two days. One thousand maggots in a compress dressing; part removed with dressing two days later. Some excoriation of skin; zinc oxide ointment applied with a simple compress dressing over the wound. Remaining maggots removed next day. All necrotic tissue had disappeared, leaving a clean granulating surface. Wound covered with skin grafts, all of which grew. Patient discharged one month after operation with wound well healed.

The maggots performed a rapid debridement of the sloughing tissue, permitting early skin graft and healing with a minimum of scar.[13]

Case 5 - W. K., age fifty-five. Hematoma and infection of wound following repair of incisional hernia. Wound opened wide; infection had produced widespread slough of subcutaneous fat. Nineteen

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[12]Case 3. Ibid (49) as Case 5.

[13]Case 4. Ibid (49) as Case 14.

days after herniorrhaphy, about 200 maggots in a compress dressing. Patient allowed to be up and about ward. On third day, patient complained of a gnawing pain in wound. Maggots removed, wound clean, all sloughing tissue having disappeared.

This case had been very slow in healing because of sloughing tissue. This was quickly cleared away by one application of maggots for three days. Rapid healing followed.[14]

The following is a summary of five case reports presented by Baer (11).

(1) Case 3 - A. W., (Figs. 25, 26, 27, and 28).

Diagnosis	Osteomyelitis of left femur
Age	6 years
Onset before admission	6 months
Admission	March 14, 1929
Previous operations	0
Operations	December 11, 1929
First insertion of maggots	January 9, 1930
Number of insertions of maggots	5
Healed	June 1, 1930
Duration	6-1/2 months
Infection	Tuberculosis and staphylococcus

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[14]Case 5. Ibid (49) as Case 47.

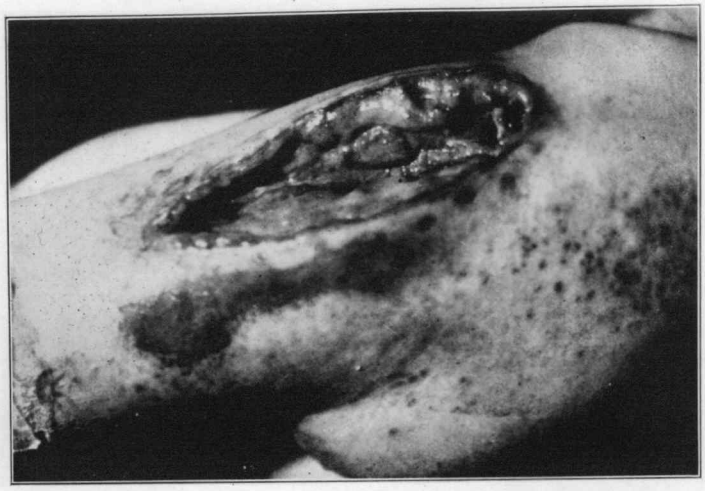

Fig. 25. During Treatment

Fig. 26. Almost Healed

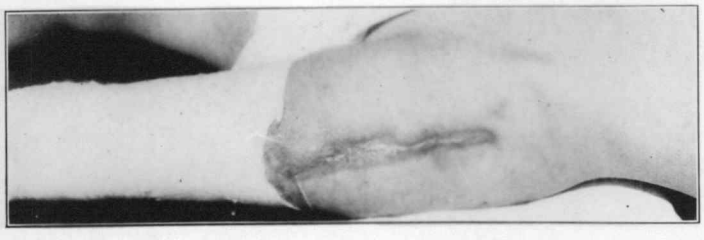

Fig. 27. After Treatment

Fig. 28. Healed

(2) Case 4 - W. C., (Figs. 29, 30, 31, 32, and 33).

Diagnosis.	Osteomyelitis of right radius
Age.	15 months
Onset before admission.	2-1/2 months
Admission.	January 25, 1930
Previous operations	0
Operations	February 12, 1930 February 26, 1930 August 26, 1930 October 10, 1930
First insertion of maggots	March 29, 1930 August 28, 1930
Number of insertions of maggots . .	4
Healed	October 30, 1930
Duration	7 months
Infection.	Staphylococcus

(3) Case 15 - F. R., (Figs. 34, 35, 36, 37, and 38).

Diagnosis.	Osteomyelitis with multiple abscesses
Age.	14 years
Onset before admission.	6 years
Admission.	November 4, 1929
Previous operations	2
Operations	November 6, 1929 March 27, 1930 September 24, 1930
First insertion of maggots	November 8, 1930 March 31, 1930 September 29, 1930
Number of insertions of maggots . .	2 12 10
Healed	May 18, 1931 Much improved
Duration	----------
Infection.	Staphylococcus

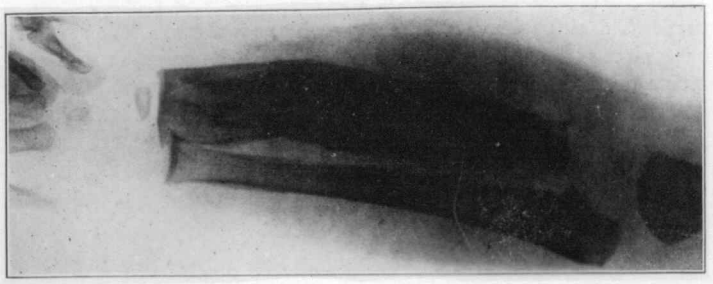

Fig. 29. Before Operation

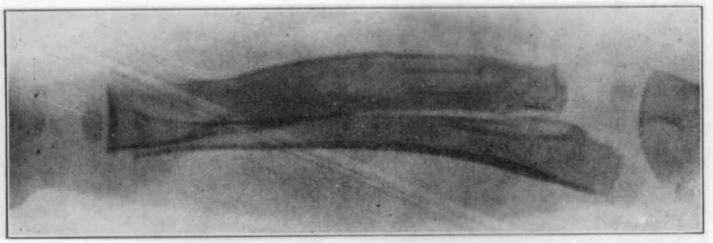

Fig. 30. After Operation

Fig. 31. During Treatment

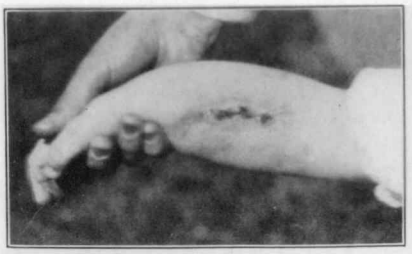

Fig. 32. Almost Healed

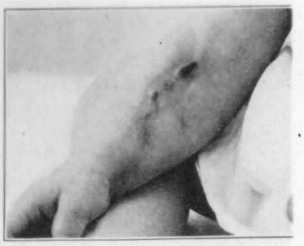

Fig. 33. Healed

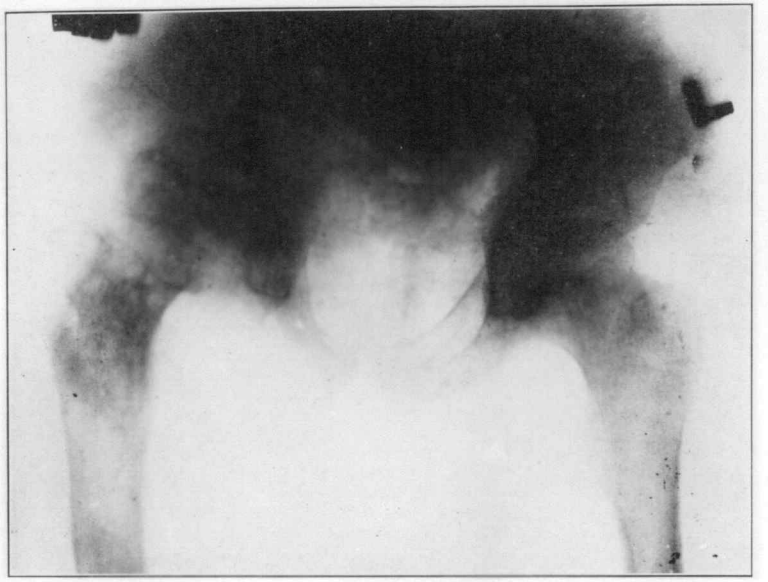

Fig. 34. Before Operation

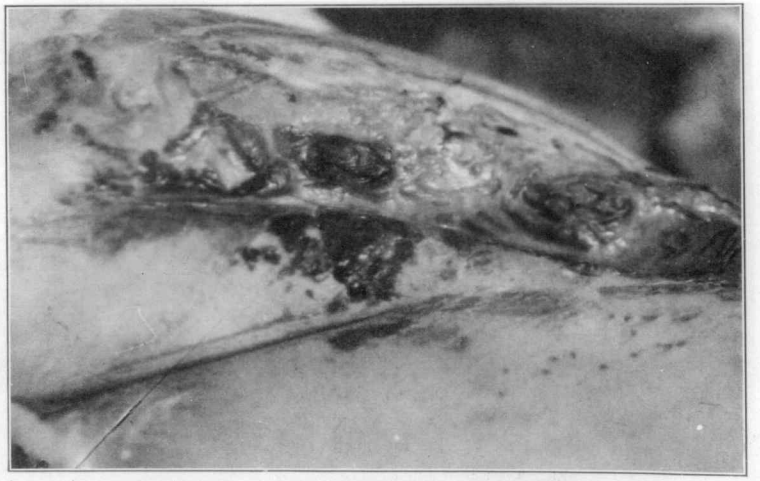

Fig. 35. During Treatment

Fig. 36. During Treatment

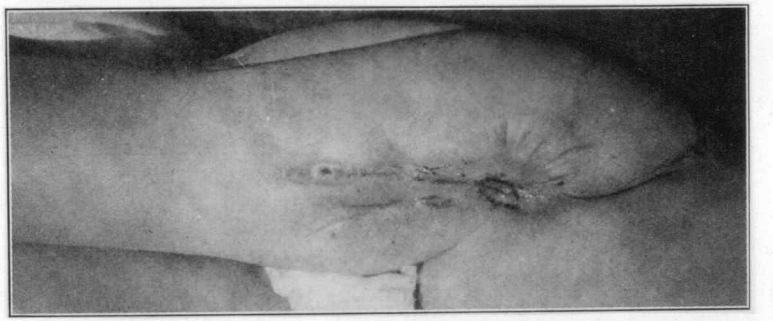

Fig. 37. Almost Healed

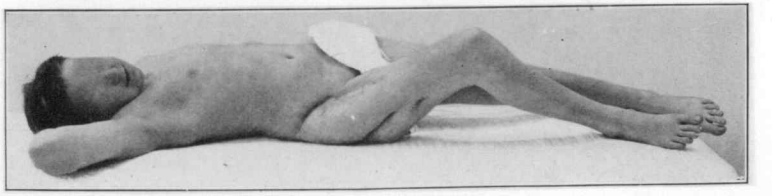

Fig. 38. Healed

(4) Case 20 - L. P., (Figs. 39, 40, 41, 42, and 43).

Diagnosis.	Osteomyelitis of both tibiae
Age.	3 years
Onset before admission.	8 months
Admission.	July 12, 1929
Previous operations	2 on each leg
Operations	July 16, 1929
First insertion of maggots	July 19, 1929
Number of insertions of maggots . .	13
Healed	Left, November 20, 1929 Right, January 17, 1930
Duration	Left, 10 months Right, 8 months
Infection.	Staphylococcus

(5) Case 28 - R. L., (Figs. 44, 45, 46, 47, 48, and 49).

Diagnosis.	Osteomyelitis of left radius
Age.	11 years
Onset before admission.	6 months
Admission.	February 24, 1930
Previous operations	1
Operations	February 26, 1930
First insertion of maggots	March 4, 1930
Number of insertions of maggots . .	9
Healed	May 23, 1930
Duration	4 months
Infection.	Staphylococcus

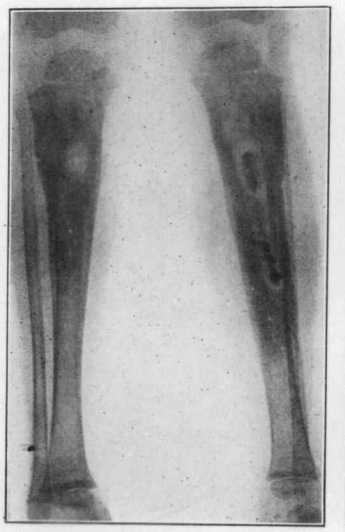

Fig. 39
Before Operation

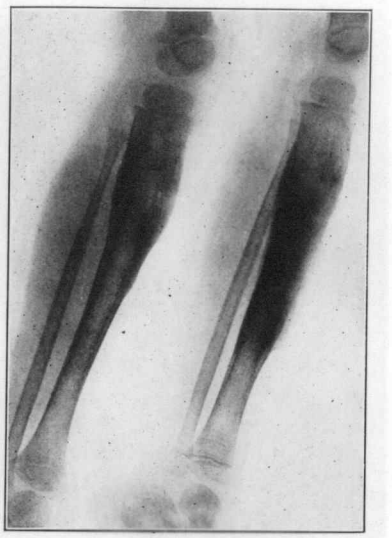

Fig. 40
After Operation

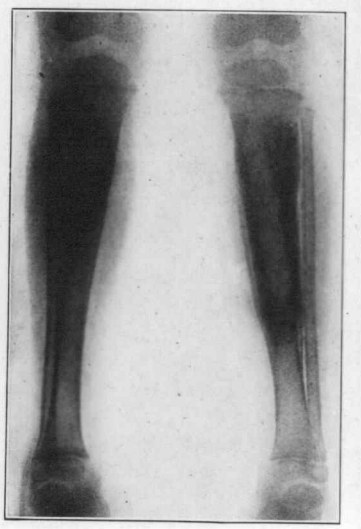

Fig. 41
After Operation

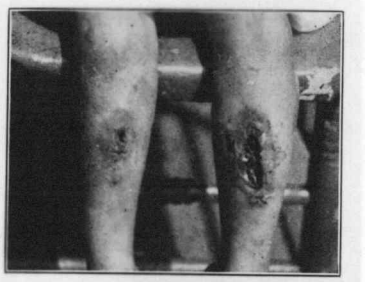

Fig. 42
During Treatment

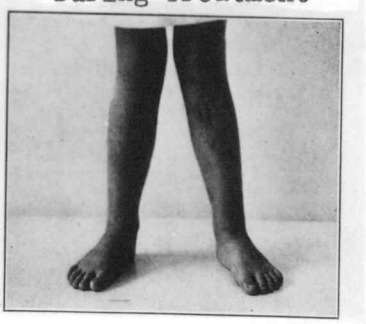

Fig. 43
Completely Healed

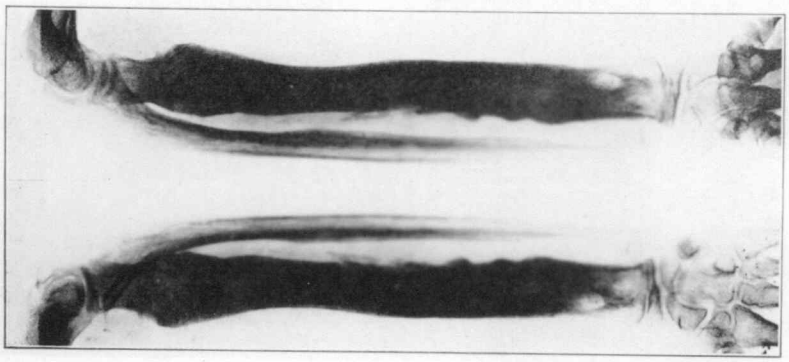

Fig. 44. Before Operation

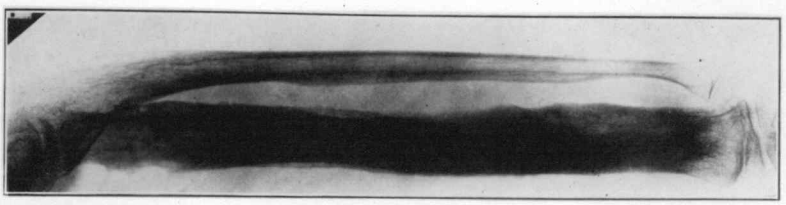

Fig. 45. After Operation

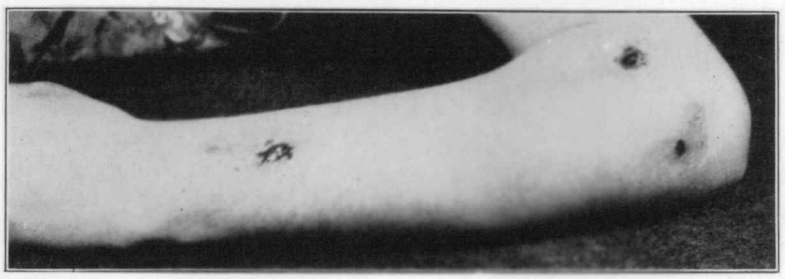

Fig. 46. Before Treatment

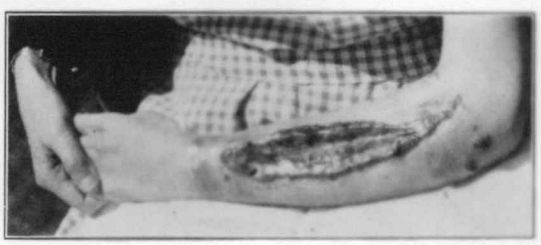

Fig. 47. During Treatment

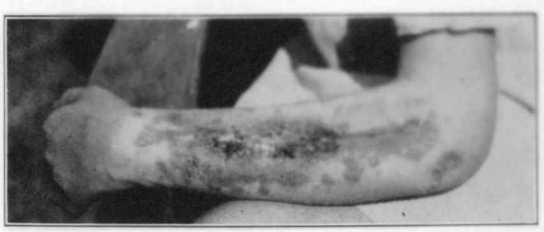

Fig. 48. Nearly Healed

Fig. 49. Healed

FACTORS EFFECTING CURE

Until very recently, the subject of the manner in which maggots act in effecting such remarkable results was one of considerable debate. There existed two schools of opinion. One school thought that the action was purely mechanical - that is, that the maggots merely fed on the purulent matter and necrotic tissue until it had all been consumed, thereby accounting for the favorable results. The other school believed that in addition to this the maggots produced some subtle biochemical substances which were largely responsible for the healing process. Recent research on the subject has shown that both points of view were correct.

Among the important beneficial actions are the following:

1. Since maggots feed upon decaying and necrotic tissue, and so remove it from the wound, conditions for bacterial development are unfavorable.

2. Baer (11) states:

> Maggots, by their digestive action, clear away the minute fragments of bone and tissue sloughs caused by operative trauma in a way not accomplished by any other means. This is a tremendously valuable asset in the healing of a wound.

3. Messer and McClellan (124) state:

> We believe our observation that blowfly maggots excrete a weakly proteolytic fluid, while containing in their digestive tracts a relatively strong one, explains why they remove necrotic tissue as effectively as they do. The use in a wound of an enzyme solution sufficiently powerful to hydrolyze the dead tissue completely would be highly irritating. The maggots apparently excrete a fluid just sufficiently proteolytic to liquefy their food, while the

bulk of the digestion takes place within their digestive tracts, out of contact with the tissues of the wound. Another important factor in the "scavenger action" is that the proteolytic end-products, which would otherwise be absorbed by the patient's system, or remain in the wound to furnish a substrate for bacteria, are used by the maggots for growth, and thus rendered inert.

4. According to Stewart (183),

Destructive bacterial exotoxin liberated into the wound, is undoubtedly ingested by the larvae feeding therein, and is probably rendered inert by the direct action of the acid present in the mid-gut or by virtue of the possession of an isoelectric point at, or close to, pH 3.2; this would cause it to throw itself down into an inert compound.

5. Stewart (183) also states:

.....that blow-fly maggot ingestion of animal tissues is dependent upon two factors: (1) a mechanical tearing up of the tissues by means of the mouth-hooks, and (2) an external digestion or liquefaction by means of digestive enzymes liberated in the excreta.

He goes on to say:

.....the liquefaction of tissue is rendered possible by the excreted tryptase, provided the wound can be rendered alkaline, since this enzyme is more active at a higher pH. Such alkalinity is not wanting, however, since the excreta of blow-fly larvae is alkaline and contains ammonia.....L. sericata larvae constantly excrete small quantities of calcium carbonate through the body wall. This inorganic salt is not only of considerable importance in increasing the pH of the wound, but the calcium ions, as demonstrated by Beckhold (1929), possess a marked specificity for phagocytosis.

Stewart states further:

The rendering of the wound alkaline not only activates the excreted tryptase....., but also, is beneficial because, as is well known, dead protoplasm is acid, and any wound in which acid debris is allowed to remain suffers from a diminution of oxygen tension in the granulation tissue cells, which in turn stimulates the autolytic enzymes contained within these cells to dissolve the surrounding protoplasm. Furthermore, acid causes a swelling of the tissue cells which pushes deeper into

> the bone canals the barriers that have been erected, thereby further extending the damage already started as a consequence of an inflammatory process in a rigid tissue. Therefore, any treatment which removes or destroys bacteria and necrotic tissue, alkalizes the wound, or increases the rate of exudation is of marked benefit.

6. Slocum, et al (177) have also called attention to the important role which ammonia plays in the disinfection of wounds. They state:

> There is a change from the acid reaction characteristic of infected wounds to an alkaline reaction greater than that of normal tissue fluids, and consequently greater than the optimum reaction for the growth of organisms associated with osteomyelitis. It is well known that the growth of staphylococci or streptococci takes place best in a medium with a hydrogen ion concentration of about 6.8. Increase in alkalinity above this optimum definitely reduce the growth of such bacteria. We have noticed a relationship between decrease in bacterial counts and increase in alkalinity in osteomyelitis wounds which corroborates this.

7. Baer (11) also pointed out the importance of wound alkalinization. He states:

> After one application of maggots, indeed after the second or third day of the first application of the maggots, all wounds become alkaline. Most wounds have an acid reaction - either weak or quite strong - but it takes hardly twenty-four hours before this reaction becomes alkaline after the introduction of the maggots. I mention this fact because I believe that the alkaline reaction has a great deal to do with the sterilization of the wound and the killing of the bacteria.

8. Messer and McClellan (124) have likewise studied the problem of wound alkalinization and in regard to this they state:

> Chronic osteomyelitis wounds, healing in the presence of blowfly larvae, develop reactions more alkaline than pH 7.4, in contrast to wounds dressed only with physiologic salt solution. Under sterile conditions, each maggot is capable of furnishing at least 0.1 mg. of ammonia nitrogen per day to its surroundings, and more than this toward the end of its larval existence. The usual wound dressing in

> osteomyelitis contains at least 100 to 200 maggots, furnishing 10 to 20 mg. of ammonia nitrogen daily. One cubic centimeter of 0.1 N ammonium hydroxide solution, containing 1.4 mg. ammonia nitrogen, is capable of changing the reaction of 2 c.c. of blood serum over approximately one pH unit. Consequently, the ammonia furnished by maggots in the usual dressing would be sufficient to neutralize much of the acid exudate characteristic of inflammation and infection, and, when the latter had to some degree subsided, to account for the excess alkalinity we have observed. It should be pointed out that maggots in the presence of bacteria produce greater quantities of ammonia than when sterile (84). The excess alkalinity is probably a factor in bacteriostasis and wound healing.

9. Simmons (176) has shown that a potent bactericide is present in maggot excretions. He remarks:

> The elimination of bacteria from pyogenic infections must precede permanent healing. The remarkable bactericidal potency of the excretions against Staphylococcus aureus, hemolytic streptococci, and Clostridium welchii accounts in part for the gratifying results obtained in such infections under maggot therapy.

10. Robinson and Norwood (163) have shown that maggots take up considerable number of bacteria with their food, and as the bacteria pass along the alimentary tract they are destroyed. These investigators state:

> .....that the disappearance of bacteria between the forestomach and the intestine in the region of greatest activity of proteolytic enzymes according to Hobson (83) indicates that the bacteria are destroyed by digestion.

Stewart, on the other hand, who reported similar findings of the destruction of bacteria in the mid and hind gut of maggots, showed histologically that the bacteria were killed but not by a process of true digestion, since they had not undergone dissolution.

11. During the course of the treatment, the wound secretes a thin, serous discharge which carries out with it great numbers of bacteria, thereby decreasing the number within the wound.

12. Livingston (100, 102) in studying the therapeutic action of maggot extract (saline extraction of crushed larvae) has shown that the extract contained all the radicals, sulphydryl $(S.H.)^-$, natural allantoin, calcium, cysteine, glutathione, and in addition embryonic growth stimulating substances. He refers to the work of Hammett and Reimann (74), and Kendall, et al (95) who have reported favorably on the sulphydryl $(S.H.)^-$ group as a growth stimulating agent. He also points out that Brunsting and Simonsen (25) have confirmed the work of Hammett and Reimann, and have satisfactorily stimulated granulation in cutaneous ulcers by means of the sulphydryl containing the amino-acid cysteine. From his investigations, Livingston concluded that maggot extract is therefore a powerful growth stimulant as evidenced by the claims for each of its growth stimulating ingredients, and that it is a more powerful growth stimulant than any one of its ingredients, which were used alone as a control.

13. The massage effect produced by the vermicular motion of the maggots on the granulating areas has been found to be a beneficial action. In regard to this, Buchman (28) states:

> .....the maggots in crawling about the wound, irritate it sufficiently to stimulate rapid growth. This factor, the importance of which is great in the proper healing of extensive wounds, is possible only with the use of maggots.

14. Of most recent interest are Robinson's reports (165, 167, 168) of the discovery of allantoin ($C_4H_6N_4O_3$) and urea (NH_2-CO-NH_2) in the urinary secretions of maggots, both substances of which have been reported to have excellent therapeutic qualities. These drugs are discussed in detail in subsequent sections.

EXTENT OF USAGE

In a survey[15] by Robinson (164), it was shown that in the United States the therapeutic use of maggots was reported by 585 surgeons, and the treatment has been given in every state. The total number of cases treated was 5,684. In Canada 20 surgeons reported using the method upon 66 cases. These results are summarized in Table 5.

Robinson (164) states that maggot therapy has also been used in Australia, Brazil, Chile, England, France, Germany, Mexico, Morocco, Spain, the Sudan, and Switzerland.

CONDITIONS TREATED WITH MAGGOT THERAPY

Table 6, also prepared by Robinson, is a summary of the various diseased conditions which have been treated with maggots.

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[15] The survey was conducted by sending out 947 copies of a questionnaire to all surgeons in the United States and Canada known to have used the maggot treatment. About 64 per cent of the questionnaires were answered. The number of physicians using this method, as well as the number of cases treated, has of course increased since this survey was made; however, the data presented here is quite representative.

TABLE 5. NUMBER OF SURGEONS USING THE METHOD AND CASES TREATED IN THE UNITED STATES AND CANADA

State	Surgeons	Cases	State	Surgeons	Cases
Alabama	4	36	Indiana	20	190
Arizona	2	2	Iowa	13	28
Arkansas	2	3	Kansas	12	75
California	25	158	Kentucky	7	36
Colorado	16	127	Louisiana	4	43
Connecticut	12	139	Maine	11	112
Delaware	1	1	Maryland	9	302
Florida	14	176	Massachusetts	10	83
Georgia	10	37	Michigan	22	378
Illinois	50	614	Minnesota	11	190
Mississippi	4	21	Pennsylvania	61	736
Missouri	10	76	Rhode Island	2	6
Montana	2	2	South Carolina	4	11
Nebraska	7	50	South Dakota	2	4
Nevada	1	1	Tennessee	23	131
New Hampshire	2	4	Utah	4	24
New Jersey	20	281	Vermont	1	1
New Mexico	3	11	Virginia	7	66
New York	90	715	Washington	8	61
North Carolina	2	4	West Virginia	10	283
North Dakota	4	7	Wisconsin	22	64
Ohio	25	301	Wyoming	2	3
Oklahoma	2	7	District of Columbia	3	56
Oregon	9	28	Canada	20	66
			Total	605	5,750

TABLE 6. SUPPURATIVE INFECTIONS TREATED WITH MAGGOTS

Abscesses, chronic	Grafts (infected), bone
tubercular	muscle
	skin
Burns, with infected necrotic areas	Mastoiditis
Carbuncles	Osteomyelitis, acute
	chronic - all bones
Cellulitis	tuberculous
Decubitus, sloughing	
	Periostitis
Empyema, chronic	
	Ulcers, chronic
Felons	syphilitic
	varicose
Gangrene, arteiosclerotic	
diabetic	Wounds, infected
gas	sloughing malignant

Robinson points out that they are all of a purulent nature, and he states that maggots have generally not been found satisfactory for use in tuberculous bone lesions unless they were complicated by osteomyelitis. He further states that maggots have been used with some success for the removal of superficial, sloughing, cancerous areas, but not before necrosis occurs.

OPINIONS OF MAGGOT THERAPY

In the same survey a request was made in the questionnaire for an expression of opinion as to the merits of the maggot treatment. The results of this request are summarized in Table 7. An unfavorable opinion was expressed by 4.4 per cent of the physicians; a reserved or neutral opinion by an equal number; and a favorable opinion by 91.2 per cent.

OBJECTIONS TO MAGGOT THERAPY

The most frequent objections to maggot therapy have been that the method is time-consuming, expensive and troublesome. Some objections have been that pain and discomfort to patients were often very great, and that the nervous tension experienced by patients with vivid imaginations at the thought of allowing maggots to feed upon them far exceeded the good that the treatment may have done. A few objections have been raised as to the efficacy of the treatment itself.

TABLE 7. SUMMARY OF OPINION EXPRESSED UPON THE METHOD

	No. of Surgeons	
Unsatisfactory	8	
Not impressed	3	
Unfavorable	3	
Does not advise it	1	
Troublesome	2	
Ineffective	5	
Doubtful benefit	1	
No better than other methods	2	
Superflous	1	
Useless	1	
Total	27	---198 cases
Neutral	3	
Reserved	23	
Total	26	--- 71 cases
Good	97	
Very good	96	
Excellent	106	
Satisfactory	25	
Very satisfactory	32	
Very effective	53	
Unequalled	22	
Definite advance	10	
Very successful	17	
Valuable	29	
Startling	8	
Very gratifying	12	
Best of all	13	
Nothing but praise	4	
Very encouraging	18	
Saved amputation	1	
Fair	2	
Well pleased	7	
Total	552	---5,481 cases

Although some of these objections may have been justified during the earlier period of maggot therapy, they are now no longer important because of the many improvements in the process of culturing sterile maggots and in the application of the treatment. To reduce the cost of production of maggots, doctors living in the same vicinity rear maggots on a club plan. I believe this investigation has shown the ease and inexpensiveness with which maggots can be cultured. Pain[16] and discomfort, although never of a serious nature, have been greatly mitigated by the use of sedatives and by newer methods of applying the treatment. Since the psychological attitude of the patient is an important factor in the treatment, the fullest cooperation of the patient must be enlisted in order to derive the best results. Some surgeons believe that the method can be used more advantageously in patients of a low psychological type who are more or less indifferent to the presence of maggots. Other surgeons have expressed the belief that patients of higher intelligence and morale are the most desirable for treatment. Still other surgeons think that the method can be carried out most successfully when the patients understand and appreciate what is being done for them.

Concerning the efficacy of the treatment itself, its wide acceptance is sufficient proof of its merits.

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[16]Pain is usually the result of the exposure of nerves by maggot feeding, and to the introduction of too many maggots in the wound.

ALLANTOIN

Robinson (165) undertook the problem of determining whether maggot secretions play any part in the healing process. He did not believe that the mechanical action of the maggots in removing purulent and necrotic tissue from the wounds could entirely account for the beneficial effects. As a result of his investigations he was able to isolate allantoin in the urinary excretions of the maggots, and after some preliminary clinical tests, he found that this substance stimulated the growth of vascular granulations, cleansed the wound, and put it into a healthy, healing condition. He described the allantoin as being bland, stable, harmless, odorless, and non-staining, and in regard to it he states:

> Allantoin is a substance occurring naturally in animal tissues, and it is also widely distributed among plants. It is regarded as an excretory material, resulting from the metabolism of the cell nucleus. When the nucleus of the cell breaks down it yields nucleic acid, and gradually through a process of simplification uric acid is produced. Man and the man-like apes are unable to split uric acid any further, and it is, therefore, excreted in the urine in that form. Strangely enough, other mammals have in their tissues an enzyme called uricase, which oxidizes uric acid one step further, and the result is the formation of allantoin, a stable end product of metabolism. Many kinds of plants produce allantoin in various parts of their structure. In its new role, as a stimulator of tissue growth where development is active, the indications are that allantoin and possibly some of its related substances are more than waste products. They might be normally used in the nuclear structure of the cell.

Robinson further states:

> The production of allantoin through the disintegration of uric acid is said to be accelerated in an alkaline medium, and the intestines of maggots (through which the urinary excretions pass) have an alkaline reaction sometimes as high as pH 8.2, and the wound becomes noticeably alkaline when maggots are used.

Although the healing effects of allantoin have been recently shown in the United States by Robinson, Robinson himself points out that its use is not new, for Macalister (104) in England in 1912 reported its therapeutic value and found the substance conspicuously present in the roots of comfrey, a plant which was formerly highly regarded by the peasantry of Europe for its healing properties. Macalister also used pure allantoin solution with success externally in the treatment of chronic ulcers and internally in the treatment of gastric and duodenal ulcers.

After its isolation and identification allantoin was carefully studied and then used in the treatment of a series of cases at the Galliger Municipal and Mount Alto Hospitals in Washington, D. C., and at the Hospital for Joint Diseases in New York, New York. Synthetic allantoin[17] which was then available was used in the tests, and was applied on gauze dressings thoroughly wetted with a 0.5 per

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[17]Synthetic allantoin is prepared from uric acid by oxidation with potassium permanganate. It is a white crystalline powder of the composition ($C_4H_6N_4O_3$), which is glyoxyldiureid. Allantoin is very slightly and slowly soluble in water, 0.6 per cent being saturation. Greenbaum (67) has shown that by chemical dispersion of from 2 per cent to 5 per cent of allantoin can be incorporated in an ointment base.

cent solution and renewed daily. In regard to this study, Robinson (165) states:

> The cases in which the allantoin treatment was given were those of chronic non-healing wounds with oedematous, indolent tissues lining the wounds, poor in circulation, and discharging pus. These included cases of chronic ulcers, of failure to heal after extensive burns, also of wounds in which suppuration had already been considerably reduced, but with little granulation. After the first few treatments, small areas of shining, pinkish granulation tissue could be seen growing in the wound, followed later by a general development of granulation. The new tissue bled easily and its appearance resembled healthy tissue. In soft-tissue wounds, the allantoin treatment was found to be very effective in cleansing the wound and producing healthy granulations. In cases of osteomyelitis, the rate of healing was not always found to be so rapid as when maggots were used initially. There is no doubt that living maggots play a definite role and are especially valuable in deep seated infections since they do not only secrete allantoin, but also remove detritus and reduce the infection. It is not claimed that in such cases allantoin will entirely replace maggots.

Shortly after the discovery of allantoin was announced, and especially after it was shown to have healing properties, many chemical and pharmaceutical concerns began to prepare allantoin in various forms and presented them to the medical profession for trial. It was hoped that eventually these preparations would replace the use of maggots. As a result of much publicity allantoin was used rather extensively, and soon afterwards a few papers regarding its use began to appear in the literature (17, 36, 67, 94, 135, 165, 166, 168, 186).

Because it was desired to obtain additional information concerning allantoin, and also because there has been considerable

controversy in regard to the use and therapeutic value of this drug, a questionnaire was sent to approximately 125 physicians and investigators, many of whom were known to have had rather extensive experience with allantoin. Through the courtesy of these men, a large amount of information was obtained. More detailed information, as well as a number of case reports, was obtained through personal communications, and through several local physicians who cooperated with the writer in this phase of the study.

The following were some of the questions asked in the questionnaire:

(1) For what conditions have you used allantoin?

(2) How was the allantoin used?

(3) Name the brand used.

(4) Give the approximate number of cases treated.

(5) For what other conditions would you recommend allantoin?

(6) What is your opinion of allantoin?

Extent of Usage

Like maggot therapy, allantoin is also being widely used. Its use was reported by physicians in every state in the United States as well as in Canada and several foreign countries. In regard to the use of allantoin, Robinson (168) states:

> The amount of allantoin being used medicinally in its various preparations is rather surprising. According to authentic sales reports, about 300,000 grams of synthetic allantoin crystals are now being produced annually. This amount would

make over 140,000 pints of 0.4 per cent solution, the usual concentration used clinically, or over 500,000 ounces of 2 per cent ointment.

Conditions Treated with Allantoin

Table 8 is a summary of the various diseased conditions which have been treated with allantoin. Most of these are the same infections which have been treated with maggot therapy.

Other Conditions Recommended for Treatment

Table 8 includes all of the conditions which were recommended for treatment with allantoin. Whatever conditions one physician recommended, there were always other physicians who had treated them.

Allantoin Preparations Used

Allantoin was first used entirely in the form of an aqueous solution, but now it is prepared in crystalline form, in solution, in ointment, and in various surgical jelly preparations. One concern has prepared a tablet form in combination with okra for the treatment of gastric and duodenal ulcers.

The ointment and jelly preparations are generally used in ambulatory cases, whereas the solutions are mostly used in hospitalized cases. However, this really depends upon the condition of the patient and the character of his wounds. Many patients are ambulatory under treatment with allantoin solution.

TABLE 8. DISEASED CONDITIONS TREATED WITH ALLANTOIN

Absecces, soft tissue
chronic

Acne

Burns, first, second and third degree
sunburn
X-ray

Carbuncles

Decubitis

Eczema

Empyema, chronic thoracic
tuberculous, with bronchopleural
pleurocutaneous fistulas
recurrent chronic non-tuberculous

Felons

Fistulas

Gangrene, gas
diabetic

Hemorrhoids, ulcerated external thrombotic

Osteomyelitis, acute
chronic
tubercular

Ozena

Pruritis ani

Rhinitis, chronic
atrophic

Sinuses, tubercular

Ulcers, chronic
corneal
diabetic
duodenal
peptic
trophic
varicose

Wounds, indolent
slow healing

Most of the physicians reported using a 0.4 per cent aqueous solution of allantoin, some a 0.5 per cent aqueous solution, a few both concentrations, and several a 2 to 5 per cent ointment. Some physicians reported using both aqueous solutions and ointments.

Detailed literature describing the various forms of allantoin and the types of cases in which each is indicated has been published by the chemical and pharmaceutical concerns supplying it. Over 80 allantoin preparations have been advertised for medical use.

In a recent review (36) on the therapeutic value of allantoin by the Council on Pharmacy and Chemistry of the American Medical Association, the limitations of ointment preparations of allantoin were pointed out. In regard to this the Council states:

> The laboratory has expressed the opinion that the effectiveness of allantoin must be greatly reduced in ointments, because of the large amounts of inert ingredients present. This opposes a claim made by a firm in some of its advertising literature that one of the advantages of allantoin (over maggot therapy) is 'increased healing of allantoin by the special ointment base'. It is also contrary to the experience of Greenbaum (67) of the firm's technical staff, who described successful use of the ointment. The evidence for the usefulness of this dosage form requires further study.

Opinions of Allantoin

The opinions expressed on the clinical value of allantoin varied considerably. A favorable opinion was expressed by 35 per cent of the physicians, a reserved or neutral opinion by an equal number, and an unfavorable opinion was expressed by 30 per cent.

The following are some of the opinions expressed:

"Excellent adjunct."

"Not as good as maggots."

"In some cases quite good, nothing striking."

"Poor."

"Excellent."

"Valuable."

"Very serviceable."

"Have not had as good luck with this as with maggots."

"Very good."

"No gain in end results."

"Excellent but too expensive; skin gets irritated easily."[18]

"Clinically inert."

It was interesting to note in some instances that although the same conditions and approximately the same number of cases were treated with the same form of allantoin by different physicians, the opinions offered were entirely opposite of each other.

Similar results were likewise brought to view in personal

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[18] Synthetic allantoin crystals cost about $1.25 for 10 grams. Ointments and surgical jelly preparations vary somewhat in price but generally cost about $0.60 an ounce. In larger quantities the price is somewhat cheaper.

communications with physicians. The following is a typical example of these differences of opinion:

> We have used allantoin from time to time. It has no influence whatever in infected wounds. In clean wounds it occasionally increases the rate of wound healing.
>
> I used allantoin at first as a wet dressing and liked it very well, but now I am using it as an ointment in a vanishing cream base. I have used this on all types of wounds. My most spectacular result recently, was in a diabetic gangrene of a portion of the finger. Allantoin ointment removed the gangrenous material, and certainly facilitated the healing process. I think allantoin removes dead infected tissue. I have used it also in a number of carbuncle cases with startling results.

This illustrates excellently, I believe, the importance of the personal element, and is one of the main reasons for making the problem of judging the efficacy of any treatment a difficult one, especially when the treatment is new. Then too, as Holder and MacKay (89) state:

> There are so many factors in each case that it is very difficult to determine the relative efficacy of various medications. These case differences make controls impossible except in the very broadest sense.

Method of Preparing Allantoin Solution

The method used in preparing allantoin solution for clinical use is to heat a liter of sterile water to near the boiling point, then 4 grams of allantoin are added and the water is heated gently, without boiling, until the crystals dissolve. This makes a solution of 0.4 per cent. The solution should be prepared under aseptic precautions, as it cannot be autoclaved or boiled without the occurrence

of chemical change. Most commercial brands of the solution now contain a disinfectant. The solution is kept at room temperature as the allantoin easily crystallizes if chilled. Exposure to sunlight must be avoided, as it will decimate the strength of the solution.

Method of Application

Before the initial treatment, the wound is cleansed thoroughly with hydrogen peroxide followed by ether and is then washed well with sterile water or normal saline solution. Sometimes debridement is necessary. For general granulation growth, the wound is loosely filled with gauze dressings well soaked with the solution, and if desired, some of the solution may be poured into the wound. It is important to place the packing into the wound lightly, otherwise it may adhere to the tissue. A wet pack of allantoin is next laid on top of the dressings to retard drying and is then covered with a dry dressing which is fastened down with strips of adhesive plaster. In deep wounds, in which it is necessary to promote healing from the bottom upward, the solution is applied in a small packing to the base of the wound, and the sides of the wound are covered lightly with vaseline. Since healing takes place only as long as the allantoin is in contact with the wound, the dressings should be changed before they become dry. Because of this characteristic, growth can easily be controlled. Allantoin also appears to stimulate local rather than a generalized granulation, and for this reason overgrowth of granulation tissue may be readily checked.

Case Reports

The following are a few typical case reports from the treatment with allantoin.

Case 1 - A woman, aged 80, whose past history was negative, developed an ulcer in the popliteal space, nine years ago, which never healed. She had been treated at various hospitals. Skin grafts were performed, to no avail. Her chief complaints were pain and inability to walk. She disregarded the nonhealing of the ulcer, as she had thought it incurable. When first seen, there was a large, foul-smelling, indurated, infected ulcer involving the entire popliteal space. After the first application of allantoin the patient was relieved from pain. Under continuous treatment with allantoin (0.4 per cent aqueous solution) over a period of two months the ulcer had decreased to one-half its size, and it is filled with bright red granulating areas. The patient, throughout the treatment, has been up and about.[19]

Case 2 - A woman, aged 65, employed as a cleaner, developed an ulcer ten years ago, following a bruise. She had used lotions and ointments with no improvement. The leg presented the typical picture of elephantiasis with a large irregular ulcer encircling it at the junction of its middle and lower thirds. A foul-smelling discharge was present. The base was necrotic. The patient complained of sharp,

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[19] Case 1. (Reported by Kaplan (94) as Case 2.)

severe, excruciating pain in the leg, inability to walk and a continuous foul discharge. Under treatment with allantoin (0.4 per cent aqueous solution) for six weeks the ulcer on the outer aspect of the leg has fully granulated, the foul odor has disappeared, the discharge has diminished, healthy granulations are present throughout the remainder of the ulcer, the patient is free of pain, and she walks with great ease. In this case the surrounding skin became reddened. An ointment dressing was placed on the skin and the irritation subsided.[20]

Case 3 - A miner sustained an extensive injury from a crushing blow while working in a mine. There was a perforation in the inguinal region. He was treated at the Ohio State Industrial Commission, of Logan, Ohio.

The Wasserman test and the sputum were negative. With the constant application of gauze saturated with allantoin solution and allantoin ointment this lesion was completely healed after a few months' treatment.[21]

Case 4 - A young man, nineteen years of age, was burned in a forest fire over eleven years previously. The injury healed but broke down at various times over the site of the original burn. In December, 1934, he fell over a wheelbarrow and reopened the scar. He was treated at the C. C. C. camp without success and was then

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[20]Case 2. Ibid (94) as Case 3.

[21]Case 3. (Reported by Greenbaum (67) as Case 1.).

transferred to the Walter Reed Hospital. For the next eighteen months every type of treatment was applied, including two different attempts at skin grafting. The result of one graft is shown in Figure 50. During this time the injury showed signs of healing only to break down again.

On June 12, 1936 the treatment was changed to allantoin ointment. Three days later there was remarkable improvement and filling in of the injury. June 25, 1936 the record shows that the ulcer is healing and the attending physician remarks that there was more progress in two weeks than in the previous eighteen months. On August 26, 1936 the condition was practically healed (Fig. 51).[22]

Case 5 - Another patient was hospitalized March 1936, with ulcers on the right and left arms and legs, diagnosed as tuberculous. They all responded to X-ray therapy except one ulcer on right leg, which did not improve with any type of treatment (Fig. 52).

Treatment with allantoin ointment was started on June 11, 1936, followed by improvement during the past six weeks (Fig. 53). In spite of the diagnosis of tuberculosis the treatment is progressing favorably.[23]

Case 6 - L. H., male, aged fifty-years was admitted to the hospital on May 17, 1937, with severe gas gangrene in the region of the back and left leg. Patient was moribund. 10,000 units of gas

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[22]Case 4. Ibid (67) as Case 2.

[23]Case 5. Ibid (67) as Case 3.

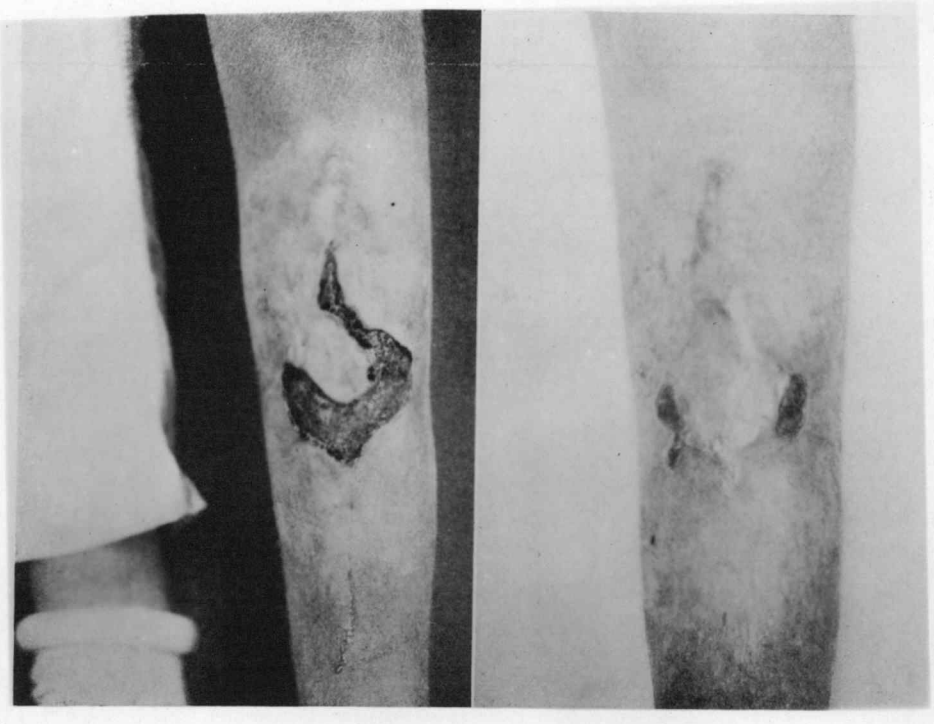

Fig. 50
Before Treatment

Fig. 51
After Treatment

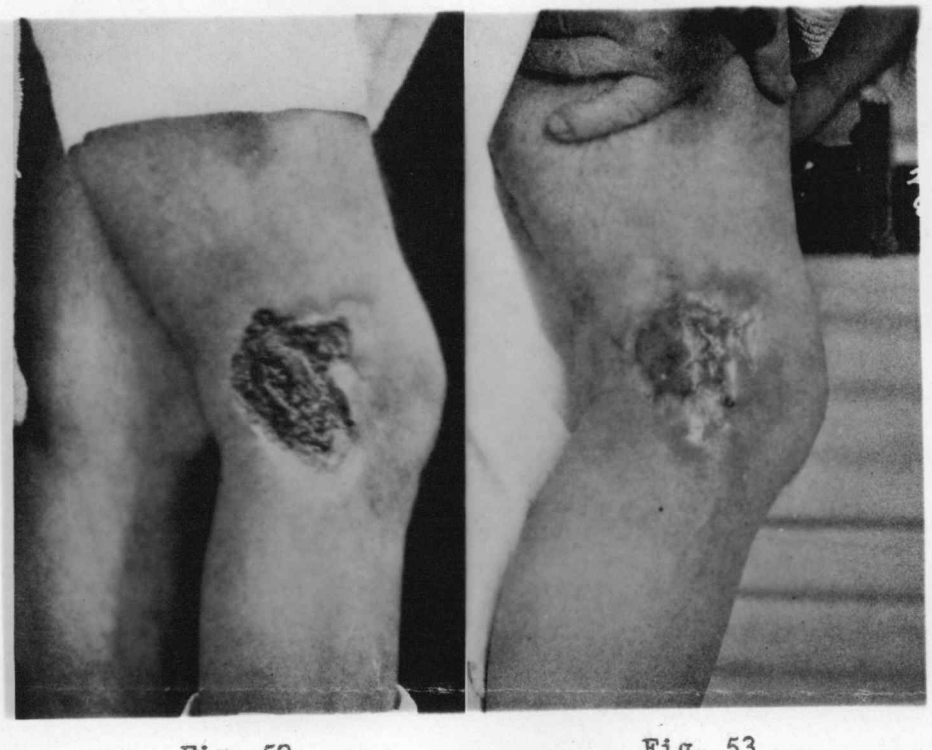

Fig. 52
Before Treatment

Fig. 53
After Treatment

gangrene tetanus antitoxin in 1,000 c.c. of normal saline solution was administered on the day of admission. Three days later another injection of 40,000 units of the antitoxin were given, and a debridement performed. The sinuses were irrigated with 1 per cent Dakin's solution. Tubes were inserted for drainage. One week later the sinuses were packed with gauze which had been saturated in a 0.5 per cent aqueous solution of allantoin. The allantoin solution packs were applied to the wounds every three hours for ten days. The necrotic tissue sloughed, and granulation tissue formed. The wounds began to heal, and recovery was uneventful.[24]

UREA

Shortly after discovering allantoin, Robinson (167) became interested in the problem of trying to isolate other substances in the maggot excretions which might possibly have therapeutic properties. He therefore continued with his investigations, and as a result found that urea, a product derived from allantoin, was also present in the excretions, and after some preliminary clinical tests it was found to produce healing effects similar to allantoin. The odor of the wounds decreased, they became cleaner, and healing progressed rapidly.

The discovery of urea led to the conception that probably allantoin is therapeutically active partly through its side chain.

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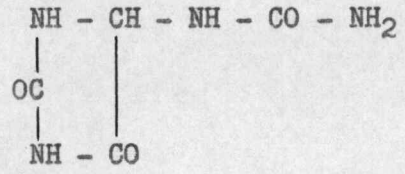
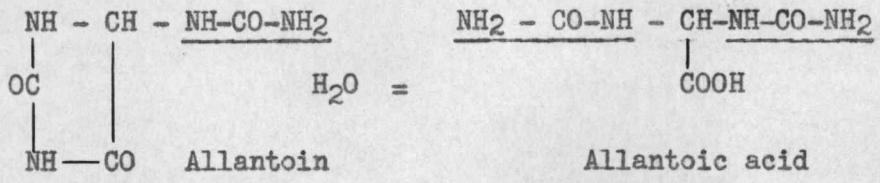
[24] Case 6. Dr. Waldo W. Ball, Corvallis, Oregon.

Robinson (167, 168) has shown that the structural chemical formula of allantoin is:



The side chain $NH-CO-NH_2$ upon hydrolysis through the addition of H from water easily forms urea, $NH_2-CO-NH_2$. In regard to this Robinson (168) states:

> Beyond the fact that allantoin and urea have healing qualities apparently similar in their action, it has not been shown that the effect of allantoin is due to the hydrolysis of its side chain to form urea. That conception merely initiated an investigation on urea. Allantoin may bring about its effects through a different set of reactions. One of the reactions of allantoin, however, is its hydration by an enzyme called allantoinase. This enzyme was discovered and named by Fosse and Brunel (1929) in the soybean and later found in a variety of plant and animal tissues, including some insects. Allantoinase reacts upon allantoin by the addition of 1 molecule of water to form allantoic acid, as follows:



> Strangely enough, whereas allantoin has one side chain NH - CO - NH_2 allantoic acid has two. Allantoinase is not known to occur in mammalian tissues.

Robinson (167, 168) describes urea as a white crystalline powder, which is bland, stable, odorless, and non-toxic, except in enormous quantities, and in regard to it he states:

> According to Marshall and Davis (1914) and Fearon (1926) urea is found in all the organs and tissues of the body. It occurs in approximately uniform concentration except in adipose tissue in which it is lower, and in renal tissue in which it is higher. The normal urea content of the blood is about 0.018 per cent. A rise or fall in the urea concentration of the blood and lymph is accompanied by a corresponding change in that of the tissues. The cells are able to absorb large quantities of urea as readily as small and the diffusion of it to the various parts of the body occurs rapidly. Marshall and Davis also refer to the work of Gryns (1896), who states that solutions of urea readilypermeate the membrane of all kinds of cells; that when cells are placed in urea solution the concentration within, at once becomes the same as without; and that urea can never cause hemolysis. On the one hand, it appears that the tissues are perpetually supplied with urea, which in sufficient concentration is a potential stimulator of healing processes. On the other hand, the existence of chronic purulent lesions indicates that the 0.018 per cent concentration of urea in the blood is incapable of promoting healing as do external applications of 2 per cent. Either the concentration in the blood is insufficient or its effectiveness is reduced by conditions in the wound. The effect of the 0.018 per cent concentration of urea on the normal growth and reparative processes of tissues remains to be investigated. The low concentration maintained in the tissues and the constant excretion of a large excess indicate an equilibrium at that point.

After being isolated and identified, urea was likewise carefully studied and then through the cooperation of a large number of physicians and surgeons it was used in the treatment of a series of cases. Synthetic urea[25] which was then available was used in

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[25]Synthetic urea is prepared commercially from the three gases nitrogen, hydrogen and carbon dioxide as follows: Nitrogen which is obtained from the atmosphere is combined with hydrogen obtained by passing steam over hot coke in the presence of a catalyzer which removes the oxygen. The N and H combine to form ammonia, NH_3. Under high pressure and low temperature ammonia is liquefied and then combined with carbon dioxide, CO_2, to form urea, $NH_2\text{-}CO\text{-}NH_2$. It is of historic interest here that urea was the first organic substance to be synthesized. This achievement was accomplished by Wohler in 1828. The cost of urea is considerably less than allantoin. It can be purchased in 1-pound bottles at 50 cents and in 20-pound containers at 18 cents a pound. Inlarger quantities it is even cheaper. In 100-pound moisture-proof sacks the price is as low as 5 cents a pound.

the tests, and like allantoin was applied on gauze dressings thoroughly wetted with a 1 per cent solution and renewed daily. The 1 per cent concentration was changed early in the work to 2 per cent, and this strength was used in the majority of the tests. In regard to this study Robinson (167) states:

> A considerable range of purulent conditions have been treated, such as varicose and diabetic ulcers, carbuncles, suppurating X-ray burns of long duration, extensive infected heat burns, intraoral infections, osteomyelitis and certain skin infections. From the results obtained, the urea treatment can be stated to produce in general, a conspicuous cleansing of the wound, with a lessening of the foul odor through the removal of necrotic material; a reduction in the pyogenic infection; and a rapid development of granulation tissue. The response of suppurating lesions to the urea treatment can sometimes be observed after two or three days' treatment, when cleansing of the wound with healing appears to be already started. As healing progresses, indolent tissues become pink or red and begin to fill the wound. The increased blood supply to the part is evident in the hyperemic color and the tendency of the wound to bleed readily. The use of a sedative, which had been given previous to the urea treatment became unnecessary, in some cases, pain being relieved during the treatment.

As in the case of allantoin, the announcement of the healing properties of urea stimulated considerable interest, and before long many chemical and pharmaceutical concerns began to produce it especially for medicinal use. Like allantoin too, it was hoped that urea would eventually replace the use of maggots. As a result of the interest in urea, it also received rather extensive use, and within a few months several papers regarding its clinical utilization were published (20, 56, 89, 98, 129, 167, 168).

Because it was desired to obtain additional information concerning urea, and also because, like allantoin, there has been considerable controversy regarding the use and therapeutic value of this drug, the same questions asked in regard to allantoin were asked about urea in the questionnaire which was sent to the physicians and investigators who had used allantoin. The majority of these men were also known to have had rather extensive experience with urea.

Like allantoin, more detailed information, as well as a number of case reports, was obtained through personal communications and through several local physicians.

Extent of Usage

Like maggot therapy and allantoin, urea is likewise being used widely. Its use was reported by physicians in every state in the United States, as well as in Canada and several foreign countries. In a personal communication with Dr. William Robinson of the United States Bureau of Entomology in Washington, D. C., the writer was informed that it has been estimated from authentic sales reports that enough allantoin and urea have been sold in the last three years to treat about 200,000 cases. In another communication with Dr. Eaton M. MacKay of the Scripps Metabolic Clinic in LaJolla, San Diego, California, the writer was told that this clinic alone had used over a ton and a half of urea since 1937.

Conditions Treated with Urea

Table 9 is a summary of the various diseased conditions which have been treated with urea. Most of these infections are the same as those treated with maggot therapy and allantoin.

Other Conditions Recommended for Treatment

Table 9 includes all of the conditions which were recommended for treatment with urea. Like allantoin, whatever conditions one physician recommended, there were always other physicians who had treated them.

Urea Preparations Used

Unlike allantoin, the number of urea preparations is extremely low. At the present time there are only four which are known to have been made. These are as follows:

(1) Synthetic crystals.

(2) 2 per cent aqueous solution.

(3) 2 per cent urea in a greaseless ointment base.

(4) 5 per cent urea in a greaseless ointment base.

Other urea preparations are under investigation.

Most of the physicians reported using a 2 to 5 per cent aqueous solution of urea, some a 2 to 20 per cent aqueous solution, a few a 40 per cent aqueous solution, and several urea crystals. Some physicians also reported using a 1 to 5 per cent ointment of

TABLE 9. DISEASED CONDITIONS TREATED WITH UREA

Abscesses, chronic
soft tissue

Acne, vulgaris

Adenitis, cervical

Angina, Ludwig's

Burns, first, second, and third degree
suppurating X-ray
extensive infected heat

Carbuncles

Carcinoma, sloughing
infected necrotic
odors from sloughing

Cellulitis

Decubitis

Dermatitis

Discharges, foul, from cervix due to malignancy

Eczema

Empyema

Felons

Fistulas, following amputation

Gangrene, gas
diabetic

Infections, chronic
following surgery
general
intraoral
pyogenic
superficial

Inflammations

Mastoiditis, acute

Meningitis, streptococcic

Osteomyelitis, acute
chronic

Otitis media, acute
chronic

Sinusitis, maxillary

Ulcers, chronic
diabetic
trophic
varicose with skin grafting
varicose without skin grafting

Wounds, dirty granulating
following trauma
indolent
infected
operative - secondary infection
operative with drainage
sloughing
slow healing

urea, a few a 5 to 15 per cent ointment, and some reported using both aqueous solutions and ointments.

Apparently most of these preparations were made up by the physicians themselves, or under their direction, since, as stated above, there are only four urea preparations available on the market. Other preparations than those mentioned above will undoubtedly be announced from time to time. In regard to urea preparations Robinson (167) remarks:

> In a greaseless ointment base, urea is giving excellent results in the treatment of ambulatory cases and under conditions where saturated gauze dressings would be uncomfortable. A greasy base should be avoided as it would interfere with the action of urea. A vanishing cream or greaseless type of ointment is satisfactory. The urea is easily combined with the ointment by thorough mixing, and as much as 15 per cent of urea can be added in this way. A fairly firm type of ointment should be used, as the urea tends to soften it. The suggestion has been made that urea added to surgical lubricating jelly and put in collapsible tubes might be found serviceable and not subject to contamination when used by patients. The addition of 15 per cent of urea is easily made and apparently does not affect the characteristic properties of the jelly. In both the ointment and the jelly the urea goes at once into solution in the water which is present.

Although urea has been used quite extensively in ointment form, some believe that the effectiveness of the urea is greatly reduced in such preparations. However, clinical evidence regarding this point is still wanting.

Opinions of Urea

The opinions expressed on the clinical value of urea were considerably more favorable than those expressed on allantoin. A

favorable opinion was expressed by 76 per cent of the physicians; a reserved or neutral opinion by 8 per cent; and an unfavorable opinion was expressed by 16 per cent. The following are some of the opinions expressed.

"Quite sufficient in some cases."

"Proved no better than other methods."

"Excellent."

"Poor."

"Valuable."

"In some cases quite good; nothing striking."

"Good dressing material."

"The best treatment of all, especially when combined with azochloramid."

"Good."

"Very serviceable."

"Not a specific or cure all. Useful in pyogenic infections and dirty wounds where there is sloughing."

"Clinically inert."

"Not as good as maggots."

Here too, as with allantoin, it was of interest to note in some instances that although the same conditions and approximately the same number of cases were treated with the same form of urea by different physicians, the opinions offered were entirely opposite of each other.

Method of Preparing Urea Solution

Urea is very soluble in water and a solution may be prepared very quickly. A concentration as high as 40 per cent is possible. Sterile distilled cold water is used; sterile, because many bacteria contain the enzyme urease, which breaks down urea and releases ammonia; and cold, because water much above body temperature would likewise cause a chemical change.

Method of Application

Urea is applied in exactly the same manner as was described for the application of allantoin.

Like allantoin, urea also appears to act only while in contact with the tissues, and healing does not persist. It also seems to exert a local effect only, and for that reason it must be applied directly to the affected tissues.

Robinson (168) has pointed out that in the treatment of extensive secondary infections of the skin following injury by poisonous plants, insect attack, heat burn or sunburn, the ordinary methods of local treatment are not feasible. He suggests that a more effective method of application would be to immerse the body in a warm bath of urea. Since urea causes a cooling effect when being dissolved, allowance should be made for this in setting the temperature of the bath. Regarding the strength of the urea bath Robinson (168) states:

> As there is a wide range in the concentration of urea that can be used effectively, no exact amount appears to be necessary when making up a solution in a tub. Two double handfuls of crystals have been found to make a satisfactory concentration.

Because commercial urea is sold at such a low price this method of treatment is not expensive, and is recommended in generalized skin conditions.

Case Reports

The following are a few typical case reports of treatment with urea.

Case 1 - S. A., female, aged twenty-four years, had chronic osteomyelitis of the right femur for three years with failure to heal and a fistula developed. This discharging sinus closed at intervals only to reopen with renewed suppuration. On January 15, 1936, treatments with urea were begun. The sinus was irrigated several times daily with 2 per cent urea solution. Within one week the fetid odor ceased and the discharge was considerably reduced. The sinus began to close and in six weeks was complete. X-ray films which previously showed small fragments of sequestra now show no evidence of osteomyelitis. To date the wound has remained healed.[26]

Case 2 - A white married woman fifty-five years old had varicose veins for twenty-five years. About eighteen years ago she underwent a surgical operation with partial relief for a few years.

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[26]Case 1. (Reported by Robinson (167) as Case 1.)

Following one or two falls she developed bruises then congestion and subsequent infection with much suffering. In February, 1936, the patient was treated with 2 per cent urea solution, the gauze dressings being kept moist. This afforded instant relief from pain with complete healing in five or six weeks. Recent reports indicate no recurrence.[27]

Case 3 - H. T., aged thirty eight years, male, presented a diabetic ulcer of left foot, 5 cm. in diameter and 1.5 cm. deep. The ulcer had been refractory to every type of treatment. In June, 1935, urea treatment was given and in four weeks the ulcer was reduced to half its size. Two months later it was entirely healed and has remained healed to date.[28]

The following case is interesting from the standpoint of control.

Case 4 - O. W., aged nineteen years, male. On February 22, 1936, the toes of both feet were markedly inflammed, with purulent and watery discharge; considerable pain for the past week and unable to walk in shoes. The right foot was soaked for a half hour three times daily with a 2 per cent urea solution and the left foot was used as a control. In two days pain and discharge of the right foot subsided, the untreated left foot was growing worse. In one week the discharge ceased in the treated foot and the toes appeared healthy and pinkish. Treatment was continued for an additional week

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[27]Case 2. Ibid (167) as Case 2.
[28]Case 3. Ibid (167) as Case 4.

when the foot appeared healed; but no improvement was apparent in the untreated foot which is now lame. Began to treat the second foot, which healed similarly in two weeks. There are no indications of recurrence.[29]

Case 5 - In postoperative inguinal hernia wounds in obese individuals where so-called "stick abscess" develops and there is a low resistance of the tissues, a 2 per cent urea solution placed deeply, markedly expedited the formation of firm granulations. In postoperative abscess located deep in the gluteal folds and fat pad, the urea solution was injected deeply and early with gratifying results.[30]

Case 6 - C. H., aged forty years, male laborer, had an amputation of the index finger for crushing injury. The wound later broke down with purulent discharge and cellulitis of the hand and arm for some time. Wet urea dressings caused cessation of discharge in forty-eight hours and was followed by rapid clearing of the condition with closure of the wound by granulation.[31]

Case 7 - B. H., aged forty-six years, female, had radium and X-ray treatment in unknown quantities for carcinoma of the cervix. A hysterectomy was followed by recurrence in the vaginal vault with sloughing and very foul odor. Tampons of urea solution caused separation of the necrotic areas, with marked diminution of odor and pain.[32]

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[29]Case 4. Ibid (167) as Case 5.
[30]Case 5. Ibid (167) as Case 6.
[31]Case 6. Ibid (167) as Case 10.
[32]Case 7. Ibid (167) as Case 11.

Case 8 - J. S., a youth, aged 17, injured the lower third of the left tibial crest in a football game and a trophic-like ulcer developed on the skin. Prolonged treatment with several commonly employed bactericidal agents failed to be effective, and the margin of the ulcer became further undermined with progressive enlargement. Ten days' application of urea crystals eliminated all infection, giving a healthy granulating base which was successfully grafted with Thiersch grafts. This urea (8 per cent) was used as a wet dressing with 90 per cent successful growth of grafts and complete epithelization. The patient was discharged home cured twenty-one days from the onset of treatment.[33]

Case 9 - On July 6, 1936, a resection of a blue dome cyst of the right breast was done on a woman forty-five years of age; the breast was not resected. Patient went home in ten days and later reported a tumefaction in the region of the operation. The mass was incised, foul smelling pus was evacuated; drainage was instituted. The pus drainage subsided in a couple of weeks but a sinus remained with a gaping wound that did not heal. Various agent were tried and the patient was told an operation would have to be done to stimulate wound repair. However, the wound was filled with urea crystals and a dressing put over the wound and the patient was instructed to instil ten per cent urea solution into the wound several times daily. The wound began to heal very rapidly and closed entirely within three weeks.[34]

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[33]Case 8. (Reported by Holder and MacKay (89) as Case 1.)
[34]Case 9. (Reported by Bogart (20) as Case 4.).

Case 10 - E. P., aged 43, had a hornifying squamous cell carcinoma of the nose, with complete destruction of the external nares. On March 16, 1937, considerable liquefactive necrosis was present. Urea was applied. By April 2 the slough, necrosis and pus had disappeared.[35]

Case 11 - B. A., aged 17, presented the complaint of cervical adenitis of two weeks' duration. The area became fluctuant and was incised, with the release of much pus. Irrigation was done three times a day with a 2 per cent solution of urea. Purulent discharge stopped in two days, with rapid subsidence of fever and uneventful recovery.[36]

Case 12 - P. S., aged 34, had a history of cervical adenitis of one weeks' duration, following erysipelas. Spontaneous opening into the canal of the ear occurred. The ear was irrigated aily with a 2 per cent solution of urea. Drainage stopped after four weeks.[37]

Case 13 - O. B. presented a history of chronic maxillary sinusitis of many years' duration. Pus was washed from both maxillary sinuses. A series of four irrigations with a 2 per cent solution of urea gave no relief. Definite relief was obtained from operation on both antrums.[38]

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[35]Case 10. (Reported by Lewy (98) as Case 4 under Carcinoma).
[36]Case 11. Ibid (98) as Case 1 under Cervical Adenitis.
[37]Case 12. Ibid (98) as Case 2 under Cervical Adenitis.
[38]Case 13. Ibid (98) as Case 1 under Maxillary Sinusitis.

Case 14 - A. H., aged 17, presented a history of granulations and foul discharge from the right ear of many years' duration. Treatment consisted of the removal of polyps and granulation followed by the instillation of a 2 per cent solution of urea. At the end of six weeks the discharge was still foul and there was no other improvement in the ear.[39]

Case 15 - J. L., aged 50, presented a history of streptococcic meningitis. Treatment consisted of extensive mastoidectomy, with uncovering of the middle and posterior fossae. On the fifth postoperative day the wound was wide and gaping, with much necrotic material at the edges and foul discharge. Dressings saturated with a 2 per cent solution of urea were applied. The odor disappeared in one day, all necrotic tissue disappearing in two days. The wound is healing rapidly at the time of writing.[40]

In discussing the therapeutic value of urea, Robinson (167, 168) states:

> The peasantry of Europe and similar classes throughout the world have for ages availed themselves of the healing properties of urine, and are today still using urine on wounds for that purpose. Frequently in the folklore and sometimes in essays and historical writings references have been made to this practice. Such statements have been traced through medieval times back to the writings of Cato, 175 B. C. Records have been found tending to show that even the ancient Babylonians, about 800 B. C., indulged in the same practice. Since the main constituent or urine, next to water, is urea, in about 2 per cent concentration, this ancient remedy for non-healing wounds now appears to be basically sound.

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[39]Case 14. Ibid (98) as Case 3 under Otitis Media.
[40]Case 15. Ibid (98) as Case 1 under Mastoid Wounds.

Robinson goes on to say:

> It is unfortunate that urea is generally associated with animal excretions. Consequently the lay attitude toward urea, even as a treatment for external wounds, might be difficult to overcome. As a matter of fact, urea is of common occurrence in plants, some of which are used as food. It so happened that Fourcroy and Vauquelin (1798), who gave urea its name, first found it in urine, and they called it "uree," thus stigmatizing it in public opinion. If this substance had first been isolated in spinach, in which it occurs (198) its name would have been something entirely different and it would no doubt be regarded without prejudice.

Clinically, synthetic urea is used, and it has no connection whatever with animal excretion. The process of preparing synthetic urea was described above.

Because of the unsavory association in the minds of most people of the term urea with urine, Fantus (46) has suggested adopting its chemical term, carbamide.

FACTORS EFFECTING CURE IN THE TREATMENT OF DISEASES WITH ALLANTOIN AND UREA

The reports of the remarkable healing properties of both allantoin and urea also have led to much discussion, and some investigation regarding the manner in which these drugs produce their beneficial results. Careful clinical studies, however, are few and far between, and because of this fact there are differences of opinion on the problem. Of course, these drugs are still new, and with further investigation some of the controversial points will, no doubt, be clarified.

Allantoin is described as a cell proliferant, and has been observed to stimulate the growth of vascular granulations, to cleanse the wound, and to put it in a healthy, healing condition. Beyond this, very little is known of its therapeutic properties.

Robinson (167) states:

> In 1912 Calkins et al (29), in testing the effect of various metabolic substances on the rate of cell division of the protozoan Actinobolus radians, found that when the seasonal vitality of the cell was high allantoin did not increase its rate of division, but when its vitality was low allantoin had a marked stimulating effect, causing the cell to reproduce nearly twice as fast as the checks. From the present and previous (165) investigations, allantoin and urea are apparently alike in activating the indolent, edematious tissues of purulent lesions and promoting development of granulations, a characteristic somewhat similar to that found by Calkins.

In regard to treatment with urea, Robinson (167) also states:

> Although the pyogenic infection of the wound is noticeably reduced during the treatment, repeated laboratory tests have failed to show that the 2 per cent concentration of urea used clinically has any direct bactericidal action on the organisms involved in chronic purulent wounds. Additional tests have been made with concentrations as high as 8 per cent with similar negative results.

Robinson goes on to say:

> Removal of necrotic material from the wound is also evident during treatment. Urea, however, has no direct proteolytic action. In concentrated solutions urea acts as a solvent of non-viable material, but in the present experiments it has been used only in dilute solution. Thus the cleansing effects obtained are not attributed to any direct activity of the urea. It has been noted, on the other hand, that the urea treatment does stimulate the proliferation of the cells of the granulation tissue and the development of capillaries. A conclusion to be drawn from this is that the cleansing effects are produced indirectly through the stimulation of a vigorous growth of granulation tissue with abundant blood supply.

Holder and MacKay (89, 90) state:

> Urea in strong solution has a peculiar property of being able to "dissolve" proteins and protein substances. First noted by Spiro (180), this lytic property was not thoroughly examined until discovered independently by Ramsden (146). This "dissolving" of proteins occurs in neutral solutions or solutions of varying degrees of alkalinity or acidity.

These authors disagree with Robinson that urea directly stimulates healing and it is their opinion that the effectiveness of the urea treatment is due to the removal of the deterrents to healing. In regard to this they state:

> We have used both the solid crystals and saturated solutions. The effect on healing in all cases is probably entirely passive; i.e., the procedures have no direct influence on cell growth or epithelization but only aid this to proceed normally by removing dead tissue, dried secretions and other hindrances. In addition, we have pointed out, strong urea solutions have some bactericidal effects and an even more definite bacteriostatic influence, which is most desirable in infected wound therapy.

Holder and MacKay go on to say:

> That dilute urea solutions give a slight degree of success we cannot deny and as we have already pointed out this is probably due to the long known activity of dilute urea solutions in promoting proteolysis of the necrotic tissue (146). Support is given to this view by a recent study (80) demonstrating that dilute urea solutions are without influence upon the rate of growth of fibroblasts in tissue culture. It is true that there is some evidence (1) that dilute urea solutions produce proliferation of capillaries by sprouting and may thus aid in wound healing. However, we prefer to avoid dilute urea solutions because of the excellent medium for bacterial growth which they provide and the superior results obtained with higher concentrations.

In spite of the observations and conclusions by Holder and MacKay, dilute urea solutions are still being used, and favorable

results reported. The question still remains, what then is the mechanism of dilute urea in effecting such favorable results. Is it simply the factor of a wet pack, which many physicians claim may effect similar response; is it, as Robinson believes, due largely to the mechanism of cell proliferation; or is it, as Holder and MacKay have pointed out, due in part to the proteolysis of necrotic tissue, and probably in part to the proliferation of capillaries by sprouting; or are there still other subtle factors which may be playing an important role. These are some of the problems which need to be clarified, and which further investigation will corroborate or refute.

In studying this phase of the problem, it occurred to the writer that perhaps the decomposition of the urea by the organisms in the wound might be one of the beneficial factors in healing by the use of dilute urea. Since dilute urea solutions do not destroy bacteria, decomposition would take place. It is known that certain pyogenic organisms, as well as many others, contain the enzyme urease, which breaks down urea with the liberation of ammonia. In cystitis, for example, the urine sometimes has a noticeable odor of ammonia. If this is the case, then the ammonia, if produced in sufficient quantity, would check the acidity of the wound, a factor of importance in the healing process. The significance of wound alkalinization has been discussed in detail in the section on factors effecting cure in the treatment of infections with maggot therapy. A study to determine this factor would be of interest.

ANTISEPTIC AND BACTERICIDAL PROPERTIES OF UREA

Ramsden (146) in studying the action of urea upon proteins, observed that in a saturated urea solution no putrefaction ever takes place.

Peju and Rajat (141) were the first to make a detailed study of the bactericidal properties of urea. They also studied the polymorphism produced in bacteria by urea. They noted that on a media containing urea bacterial growth was inhibited, and on a media saturated with it no growth occurred.

Wilson (201) also studied the effects of urea on bacterial growth, and found that 8 per cent urea prevented the growth of Bacterium coli (Escherichia coli) while 1.5 to 3.5 per cent caused polymorphism.

Symmers and Kirk (187), likewise studied the effects of urea on bacterial growth, and in addition conducted clinical tests on its bactericidal action. They made a number of interesting and important findings; these are summarized by Foulger and Foshay (56) as follows:

a. Urea prevents bacteria from deoxidizing blood.

b. No growth could be obtained on tubes inoculated from twenty-four-hour agar culture of B. typhosus which had been subjected for thirty minutes to the action of a saturated solution of urea.

c. An old, putrid, tuberculous sputum, which gave, on agar, confluent growths of various organisms, was treated at room temperature with a saturated solution of urea. After fifteen minutes a loopful was smeared on agar. Only three colonies grew. After thirty minutes' action of urea only one colony grew.

d. Five cubic centimeters of blood were inoculated with B. pyocyaneus. Controls gave abundant growth, but transplants from the mixture after it had been treated with 2.5 gm. of urea for thirty minutes at room temperature were sterile.

e. A similar mixture containing 1.25 gm. of urea gave ninety-eight colonies on an agar transplant after standing fifteen minutes, while after standing thirty minutes only five colonies grew.

f. Below a concentration of 25 gm. of urea per 100 c.c. of blood there was no definite bactericidal action, but growth was delayed.

Foulger and Foshay (56), impressed with the favorable results and bactericidal properties of urea, used it for the treatment of purulent otitis media with good results, and studied the bactericidal action of half-saturated urea solution.

Holder and MacKay (89), also studied the bactericidal properties of urea, and have found that urea solutions of less than 15 per cent are not uniformly bacteriostatic and that those of less than 30 per cent not usually bactericidal. Of the common pyogenic organisms, the staphylococcus was found to be the most resistant to urea action.

Being familiar with Foulger's and Foshay's (56) study of the bactericidal action of half-saturated urea, but unaware of the work of the other investigators mentioned above, it was felt desirable to test the bactericidal action of other concentrations of urea. The concentrations known to have been used clinically were selected for study. These were as follows:

(1) 2 per cent aqueous solution

(2) 5 " " " "

(3) 10 " " " "

(4) 20 " " " "

(5) 30 " " " "

(6) 40 " " " "

Each concentration was tested against thirteen different organisms, most of which are associated with suppurative infections. The following were the organisms used:

(1) Staphylococcus aureus (official).

(2) Staphylococcus albus (I. C. Hall).

(3) Escherichia coli (Levine 1a.).

(4) Pseudomonas aeruginosa (pyocyanea) (I. C. Hall).

(5) Salmonella paratyphi (Paratyphoid A).

(6) Salmonella schottmulleri (Paratyphoid B).

(7) Eberthella typhi (Dr. Sears).

(8) Bacterium dysenteriae.

(9) Corynebacterium diphtheriae (D.B.C.F.4a).

(10) Alkaligenes fecalis.

(11) Streptococcus fecalis (Stark 22S)

(12) Streptococcus liquefaciens

(13) Streptococcus zymogenes

Technic 1

Approximately 200 c.c. of a 40 per cent solution of urea was prepared and then filtered through an L3 to 5 Pasteur-Chamberlin porcelain filter which had been previously sterilized. The filtrate was then tested for sterility. Tubes of plain broth, veal infusion broth, and 1 per cent dextrose veal infusion broth were inoculated with the filtrate and then incubated at 37° C. for 48 hours. All culture tubes were incubated at 37° C. for an additional 48 hours before being discarded. Under aseptic technic dilutions were made from the sterile 40 per cent urea solution. Each tube contained exactly 5.0 c.c. of solution. Then each tube was inoculated with a large loopful of a 24 hour broth culture of the test organism, and at intervals of 1, 2, 3, 4, and 24 hours,[41] large loopfuls of each of the urea-bacteria suspensions were transferred to appropriate broth tubes to determine viability. One of the broth tubes was inoculated directly with a loopful of the 24 hour culture of the organism to serve as a control. The tubes stood at room temperature throughout the entire period. All culture tubes were incubated at 37° C. for 48 hours, after which time readings were made. Before being discarded the tubes were incubated for an additional 48 hours. The results of these tests are shown in Tables 10, 11, 12, 13, 14, and 15.

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[41] A preliminary test was first made at intervals of 1, 2, 3, and 30 minutes with Staphylococcus aureus, but these periods of time were not sufficient to kill the test organism. The intervals of time were, therefore, changed to 1, 2, 3, 4, and 24 hours.

TABLE 10. VIABILITY AS DETERMINED BY CULTURE

Organism Used	Exposure To 2 Per Cent Urea					
	1hr	2hr	3hr	4hr	24hr	Control
Staphylococcus aureus	≠	≠	≠	≠	≠	≠
Staphylococcus albus	≠	≠	≠	≠	≠	≠
Escherichia coli	≠	≠	≠	≠	≠	≠
Pseudomonas aeruginosa	≠	≠	≠	≠	≠	≠
Salmonella paratyphi	≠	≠	≠	≠	≠	≠
Salmonella schottmulleri	≠	≠	≠	≠	≠	≠
Eberthella typhi	≠	≠	≠	≠	≠	≠
Bacterium dysenteriae	≠	≠	≠	≠	≠	≠
Corynebacterium diphtheriae	≠	≠	≠	≠	-	≠
Alkaligenes fecalis	≠	≠	≠	≠	≠	≠
Streptococcus fecalis	≠	≠	≠	≠	≠	≠
Streptococcus liquefaciens	≠	≠	≠	≠	≠	≠
Streptococcus zymogenes	≠	≠	≠	≠	≠	≠

≠, growth

-, no growth

TABLE 11. VIABILITY AS DETERMINED BY CULTURE

Organism Used	Exposure To 5 Per Cent Urea					
	1hr	2hr	3hr	4hr	24hr	Control
Staphylococcus aureus	≠	≠	≠	≠	≠	≠
Staphylococcus albus	≠	≠	≠	≠	≠	≠
Escherichia coli	≠	≠	≠	≠	≠	≠
Pseudomonas aeruginosa	≠	≠	≠	≠	≠	≠
Salmonella paratyphi	≠	≠	≠	≠	≠	≠
Salmonella schottmulleri	≠	≠	≠	≠	≠	≠
Eberthella typhi	≠	≠	≠	≠	≠	≠
Bacterium dysenteriae	≠	≠	≠	≠	≠	≠
Corynebacterium diphtheriae	≠	≠	≠	≠	-	≠
Alkaligenes fecalis	≠	≠	≠	≠	≠	≠
Streptococcus fecalis	≠	≠	≠	≠	≠	≠
Streptococcus liquefaciens	≠	≠	≠	≠	≠	≠
Streptococcus zymogenes	≠	≠	≠	≠	≠	≠

≠, growth

-, no growth

TABLE 12. VIABILITY AS DETERMINED BY CULTURE

Organism Used	Exposure To 10 Per Cent Urea					
	1hr	2hr	3hr	4hr	24hr	Control
Staphylococcus aureus	≠	≠	≠	≠	≠	≠
Staphylococcus albus	≠	≠	≠	≠	-	≠
Escherichia coli	≠	≠	≠	≠	≠	≠
Pseudomonas aeruginosa	≠	≠	≠	≠	≠	≠
Salmonella paratyphi	≠	≠	≠	≠	≠	≠
Salmonella schottmulleri	≠	≠	≠	≠	≠	≠
Eberthella typhi	≠	≠	≠	≠	≠	≠
Bacterium dysenteriae	≠	≠	≠	≠	-	≠
Corynebacterium diphtheriae	≠	≠	≠	-	-	≠
Alkaligenes fecalis	≠	≠	≠	≠	≠	≠
Streptococcus fecalis	≠	≠	≠	≠	≠	≠
Streptococcus liquefaciens	≠	≠	≠	≠	≠	≠
Streptococcus zymogenes	≠	≠	≠	≠	≠	≠

≠, growth

-, no growth

TABLE 13. VIABILITY AS DETERMINED BY CULTURE

Organism Used	Exposure To 20 Per Cent Urea 1hr	2hr	3hr	4hr	24hr	Control
Staphylococcus aureus	/	/	/	/	-	/
Staphylococcus albus	/	/	/	-	-	/
Escherichia coli	/	/	/	/	-	/
Pseudomonas aeruginosa	/	/	/	-	-	/
Salmonella paratyphi	/	/	/	/	-	/
Salmonella schottmulleri	/	/	/	/	-	/
Eberthella typhi	/	-	-	-	-	/
Bacterium dysenteriae	-	-	-	-	-	/
Corynebacterium diphtheriae	/	-	-	-	-	/
Alkaligenes fecalis	/	-	-	-	-	/
Streptococcus fecalis	/	/	/	/	/	/
Streptococcus liquefaciens	/	/	/	/	-	/
Streptococcus zymogenes	/	/	/	/	/	/

/, growth

-, no growth

TABLE 14. VIABILITY AS DETERMINED BY CULTURE

Organism Used	Exposure to 30 Per Cent Urea					
	1hr	2hr	3hr	4hr	24hr	Control
Staphylococcus aureus	/	/	/	/	-	/
Staphylococcus albus	-	-	-	-	-	/
Escherichia coli	-	-	-	-	-	/
Pseudomonas aeruginosa	-	-	-	-	-	/
Salmonella paratyphi	-	-	-	-	-	/
Salmonella schottmulleri	-	-	-	-	-	/
Eberthella typhi	-	-	-	-	-	/
Bacterium dysenteriae	-	-	-	-	-	/
Corynebacterium diphtheriae	-	-	-	-	-	/
Alkaligenes fecalis	-	-	-	-	-	/
Streptococcus fecalis	/	/	/	/	-	/
Streptococcus liquefaciens	/	/	/	/	-	/
Streptococcus zymogenes	/	/	/	-	-	/

/, growth

-, no growth

TABLE 15. VIABILITY AS DETERMINED BY CULTURE

Organism Used	Exposure To 40 Per Cent Urea 1hr	2hr	3hr	4hr	24hr	Control
Staphylococcus aureus	-	-	-	-	-	≠
Staphylococcus albus	-	-	-	-	-	≠
Escherichia coli	-	-	-	-	-	≠
Pseudomonas aeruginosa	-	-	-	-	-	≠
Salmonella paratyphi	-	-	-	-	-	≠
Salmonella schottmulleri	-	-	-	-	-	≠
Eberthella typhi	-	-	-	-	-	≠
Bacterium dysenteriae	-	-	-	-	-	≠
Corynebacterium diphtheriae	-	-	-	-	-	≠
Alkaligenes fecalis	-	-	-	-	-	≠
Streptococcus fecalis	≠	-	-	-	-	≠
Streptococcus liquefaciens	≠	≠	≠	-	-	≠
Streptococcus zymogenes	≠	≠	≠	-	-	≠

≠, growth

-, no growth

It will be noted that the 2, 5, and 10 per cent urea solutions are almost entirely without effect upon any of the organisms. The 2, 5, and 10 per cent concentrations of urea apparently killed Corynebacterium diphtheriae after 24 hours of exposure, as no growth occurred after inoculation in the broth tubes. Ten per cent urea also killed Straphylococcus albus and Bacterium dysenteriae after 24 hours of exposure, and Corynebacterium diphtheriae after 4 hours of exposure.

Bactericidal activity becomes more evident in the 20 per cent concentration, somewhat greater in the 30 per cent concentration, and quite marked in the 40 per cent concentration.

It will also be noted that the staphylococci and streptococci resist the action of urea better than the other species tested, especially Staphylococcus aureus, Streptococcus fecalis, Streptococcus liquefaciens, and Streptococcus zymogenes.

These tests were repeated under exactly the same conditions and the same results were obtained.

Another test was as follows:

Technic 2

0.1 c.c. of a 24 hour broth culture of Staphylococcus aureus was added to 5.0 c.c. of each of the concentrations of urea used in the above tests, and also added to 5.0 c.c. of sterile water to serve as a control. The tubes stood at room temperature. After 24 hours of exposure, 1.0 c.c. of each of the urea-bacteria suspensions was run into

sterile petri plates, and then 15 c.c. of molten agar which had been cooled to 45° C. was added to the plates. The plates were then covered and rotated in the usual manner to distribute the organism. The same was done for the control. When the agar had hardened, the plates were inverted and incubated at 37° C. for 48 hours, at the end of which time they were removed for observation. The control plate, as well as the 2, 5, and 10 per cent urea-plates, were beyond count. The 20 per cent plate showed one colony, while the 30 and 40 per cent plates were sterile. All plates were incubated for an additional 48 hours before being discarded. This experiment was repeated, but instead of exposing the organism for 24 hours, it was exposed for 48 hours. The results of this test were the same as those of the first test, except for the 20 per cent urea-plate which was sterile after 48 hours of exposure.

Both tests were repeated under exactly the same conditions and the same results were obtained.

The inhibitory and bactericidal properties of crystal urea were also tested. The standard Agar-Plate and Agar Cup-Plate Methods of the United States Food and Drug Administration (170) were used in these tests. The test organism was the official Staphylococcus aureus.

Technic 3

15 c.c. of agar, in each of two test tubes, was melted in a steam sterilizer and then cooled to 45° C. To each tube was added 0.1 c.c. of a 24 hour broth culture of the test organism. The

inoculated agar was then poured into two sterile petri plates and allowed to harden. As soon as the agar hardened, a small disk was cut out of the center of the agar of one plate by means of a sterile cork borer, and all cracks and crevices sealed with several drops of melted agar. The cup in the agar was then filled with urea crystals. On the surface of the agar in the other plate was placed an equal amount of urea crystals. As a control, a small amount of warmed sterile vaseline was placed on the surface of a third agar plate which had also been inoculated with the test organism. The plates were incubated at 37° C. for 48 hours and then removed for observation. Each of the urea-plates showed a zone of clear agar about 10 mm. in width around the urea crystals, indicating inhibitory properties (Figs. 54 and 55). The control showed growth adjacent to and under the vaseline, indicating no inhibitory properties (Fig. 56). To determine whether the action was germicidal or merely inhibitory, a small portion of agar in each of the clear zones was subcultured in tubes of broth. The tubes were incubated for 48 hours at 37° C. and then removed for observation. No growth took place, indicating that the action of the urea was bactericidal. The plates were incubated for an additional 48 hours before being discarded.

This experiment was repeated under exactly the same conditions and the same results were obtained.

From the tests conducted it may be concluded that urea does possess antiseptic and bactericidal properties, but these are rather mild in action.

Fig. 54

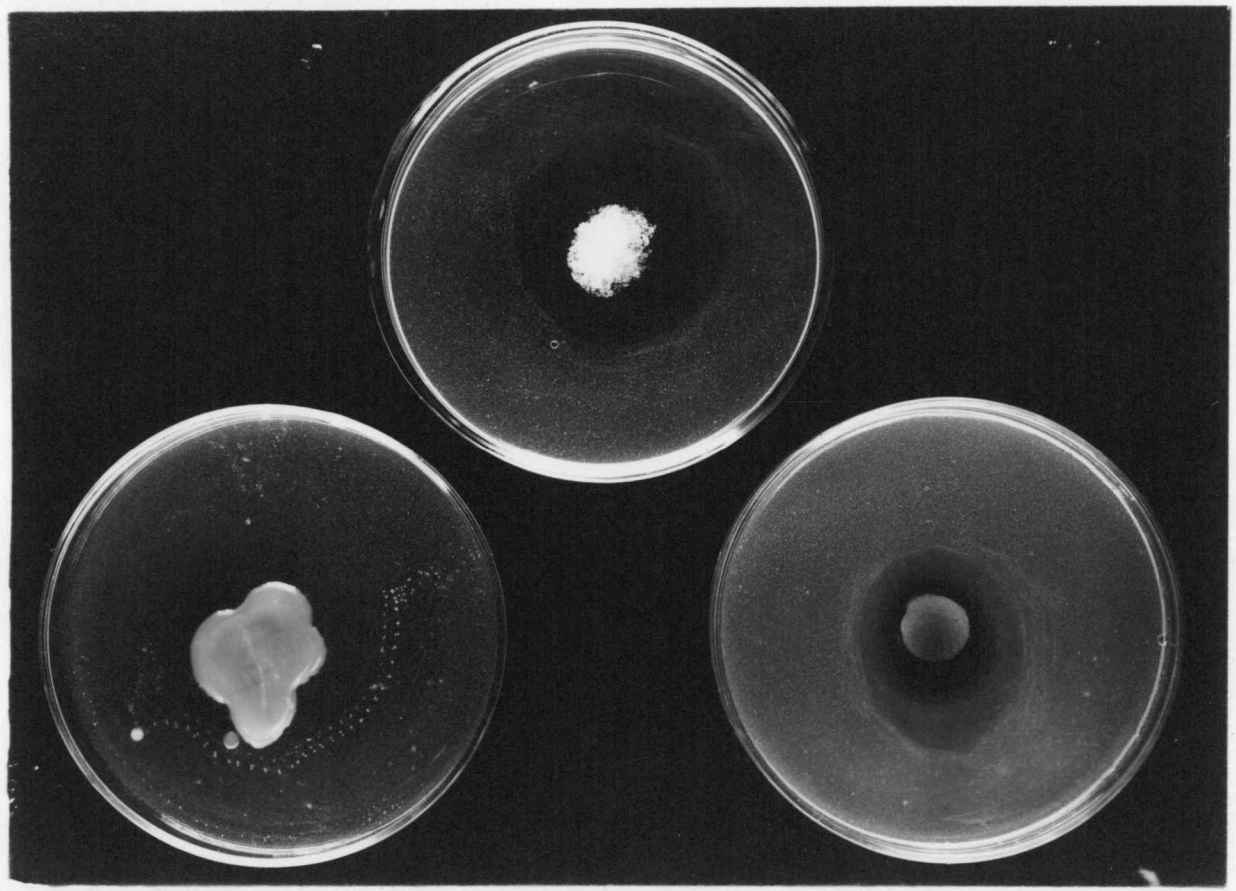

Fig. 56

Fig. 55

In regard to the bactericidal properties of urea, Holder and MacKay (89) state:

> Our experience has led us to conclude that urea in any concentration is not a really good germicide and that, although most certainly a contributing factor, bactericidal activity is not the chief influence in the usefulness of this substance for wound therapy.

SUMMARY

1. A simplified method of culturing maggots for surgical use was devised.

2. A number of the clinical aspects of maggot therapy were investigated.

3. The culture methods were investigated from three standpoints.

(1) Can growth of the entire life cycle of the fly be carried on successfully under ordinary laboratory conditons, and if so, what conditions are necessary to maintain a healthy strain of both maggots and flies.

(2) How might the culture of maggots by the method of incubation be made simpler and more economical without producing some unsatisfactory effect upon the development of the eggs, larvae, pupae, and flies.

(3) What advantages, if any, does the method of culturing maggots by incubation have over the method of culturing maggots without incubation.

4. This phase of the study, therefore, was developed as a comparison of growth in the laboratory with growth in the incubator.

5. It was found that growth of all of the stages in the life cycle of the fly can be carried on entirely in the laboratory, and the flies can lay eggs and live in the laboratory as well as in the incubator.

6. The rate of growth in the incubator under constant temperature (78° to 80° F.) and humidity (50 to 60 per cent) was no more regular than the rate of growth in the laboratory under fluctuating temperature (50° to 95°F.) and humidity (35 to 75 per cent).

7. There were no noteworthy differences manifested in fly mortality, egg-laying capacity, egg viability, or the general character of the eggs, larvae, pupae, and flies grown in the laboratory as compared with those reared in the incubator.

8. Flies exposed to a poorly illuminated environment showed no noteworthy differences in mortality, egg-laying capacity, egg viability, or general character as compared with flies kept in a well illuminated environment.

9. The problem of myiasis in wounds was also investigated. It was found that myiasis, although possibly a potential source of danger in maggot therapy, has not thus far presented a serious problem. Clinical evidence has corroborated this fact.

10. The method of treating infections with maggots, case reports, the factors effecting cure, the extent to which maggot therapy has been used, the conditions treated, opinions of maggot therapy, and objections to maggot therapy have all been discussed.

11. Allantoin and urea, two substances isolated from the excretions of maggots, and which have been shown to have therapeutic properties, were also investigated.

12. The extent to which allantoin and urea have been used, the conditions treated, allantoin and urea preparations used, opinions of allantoin and urea, the method of preparing allantoin and urea solutions, the method of application, case reports, and the factors effecting cure in the treatment with allantoin and urea have all been discussed.

13. Finally, a study was made of some of the antiseptic and bactericidal properties of urea. The concentrations of urea known to have been used clinically were selected for study. These included 2, 5, 10, 20, 30, and 40 per cent aqueous solutions. Each concentration was tested against thirteen different organisms, most of which are associated with suppurative infections.

14. It was found that the 2, 5, and 10 per cent urea solutions were almost entirely without effect upon any of the organisms tested. Bactericidal activity became more evident in the 20 per cent concentration, somewhat greater in the 30 per cent concentration, and quite marked in the 40 per cent concentration. The staphylococci and streptococci were found to resist the action of urea better than the

other organisms tested, especially Staphylococcus aureus, Streptococcus fecalis, Streptococcus liquefaciens, and Streptococcus zymogenes.

15. Crystal urea was also tested and found to have rather marked inhibitory and bactericidal properties.

CONCLUSIONS

The production of brood larvae and the culture of sterile maggots are quite simple. The varied conditions under which both flies and maggots may live, and their simple food requirements, permit their culture to be carried on economically. The cost of necessary apparatus is nominal, since the apparatus can all be home-made. In laboratories where the insulation is quite good, and where the temperature does not fluctuate too greatly and the humidity does not fall too low, special incubators for the housing of flies and maggots are unnecessary. The brood cages and larval receptacles may be kept in the laboratory. If development should fall below a certain desired rate, growth may be hastened by simple means of incubation. The culture methods are not difficult and the technic may be learned within a very short time.

In view of the evidence presented in this investigation it seems justifiable to conclude that maggot therapy has proved a very valuable therapeutic measure for the treatment of osteomyelitis and a host of other suppurative infections.

Regarding allantoin and urea, it is perhaps too early to draw any definite conclusions. In spite of their widespread use and

the many favorable reports of the healing properties of allantoin and urea, there are still many differences of opinion concerning the recommended forms and dosages of these drugs, as well as their exact therapeutic value. For this reason further investigation is indicated. Accumulating clinical evidence, however, seems to show that both allantoin and urea may find a useful place in the field of therapeutics.

Because allantoin and urea have been shown to exhibit healing properties similar to that of maggot therapy it was hoped that they would eventually replace the use of maggots. Experience thus far has shown that although these drugs have been used as successfully in certain forms of infection, in other types they were not as effective as when living maggots were used. However, as has been pointed out by many investigators, it is doubtful whether maggots will ever be completely replaced. Even those who are working along chemical lines and have been most desirous of finding a satisfactory substitute for maggot therapy suggest the use of maggots at certain stages of the infection, and feel that in certain types of diseases greater progress could be effected by employing maggot therapy in conjunction with chemotherapy. It must also be remembered that the maggot has an added advantage over any chemical, which is the maggots discriminatory power to distinguish between viable and necrotic tissue.

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