A BACTERICIDAL PRINCIPLE IN EXCRETIONS OF SURGICAL MAGGOTS WHICH DESTROYS IMPORTANT ETIOLOGICAL AGENTS OF PYOGENIC INFECTIONS[1]

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The elimination of bacteria from pyogenic infections must precede permanent healing. That such a phenomenon occurs under the influence of maggot therapy is well known. Baer, in his original report (1929), called the maggots "a viable antiseptic." Later (1931) he had the following to say: "Whatever the bacteria of the original wound, be they aerobes or anaerobes ... the amount begins to diminish from the first application of maggots." Weil and coworkers (1933) made preliminary bacteriological studies of wounds before implantation of maggots, followed by subsequent cultures at each dressing, and found a marked decrease in infection as the treatment progressed. Other workers (Buchman and Blair, 1932; Livingston and Prince, 1932; Myers and Czaja, 1931; Slocum et al., 1933) have obtained the same results. How this remarkable phenomenon is accomplished however, has been only partially shown.

Among the factors that contribute to wound disinfection are: 1. The mechanical washing out of bacteria through stimulation of drainage by the maggots; by *a*, mechanical stimulation of the viable tissue; *b*, enzymic liquefaction of the necrotic tissue; and *c*, increase of discharge through dilution by maggot excretions, or a combination of these factors. 2. The destruction of organisms in the alimentary tract of the maggots, as has been shown by Robinson and Norwood (1933). 3. The utilization of necrotic tissue as maggot food, thus rendering conditions less favor-

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able for bacterial growth. 4. The rapid development of vascular granulation tissue. It has also been suggested that the increase in pH is a factor.

The foregoing factors, although of importance, can hardly account for the almost complete sterility obtained in some infections. The excretions of surgical maggots, which are continually released into the wound, have not heretofore been shown to have bactericidal properties. Livingston and Prince (1932) claimed to have demonstrated bactericidal action with filtered extracts from bodies of crushed maggots. Their work, however, was not substantiated by sufficient evidence, and several investigators (Maseritz, 1934; Robinson and Norwood, 1933; Slocum et al., 1933) have since proved their theory to be wrong.

The material used in this investigation is not an extract and is entirely different from that tried by other workers. It consists of the natural elimination products of living maggots, and tests with such material have not been previously reported.

The investigation has revealed that a potent bactericide is present in maggot excretions. The substance collected and used in these experiments consisted of the entire elimination products of the maggots, which included fecal matter as well as cutaneous and oral secretions. There is reason to believe, however, that the active principle is contained in the feces. Robinson and Norwood (1933) found the contents of the hind intestine of maggots to be sterile while those of the crop and stomach were heavily contaminated, and their findings have recently been confirmed by Stewart (1934). Duncan (1926) found that the feces of certain insects were sometimes sterile. The substance at present, therefore, is called "maggot excretions," for lack of a more specific name.

The preliminary work, herein presented, has been confined to tests of the bactericide on seven species of bacteria, *in vitro*. Two of these play the principal etiological rôle in pyogenic infections, and another is often a dangerous and sometimes fatal secondary invader.

All organisms were exposed to the material at a temperature of 37°C., unless otherwise designated. The maggots used were

those of *Lucillia sericata*, the species most commonly employed in wound treatment.

TECHNIC OF COLLECTING EXCRETIONS

Most of the material used was collected from three-day-old non-sterile maggots that had been reared on decayed beef, in order to simulate as closely as possible conditions encountered in maggot therapy. An excess of eggs was placed on the meat, thus increasing the ratio of maggots to food, which facilitated their removal when ready for use. The specimens were mechanically removed by raking them from the residue with a spatula, without the use of water. They were placed in a fine-mesh sieve over a funnel fixed into the neck of a flask. A fine-mesh wire gauze fastened over the top of the sieve by means of a tight-fitting metal collar prevented the maggots from escaping. Distilled water was sprayed occasionally through the top with an atomizer, in order to keep the maggots moist and actively excreting, and also to wash the excretions from the maggots through the sieve into the collecting flask.

The amount of water used varied, and thus, also, the dilution of the excreta. Usually the amount collected was approximately that of the water applied. Collection usually extended over a period of from two to four hours and from 25 to 50 cc. of water were ordinarily used. The quantity of maggots employed varied, but averaged approximately 150 to 200 cc. (measured by jarring the specimens down in a 250-cc. graduated cylinder). After collection, the material was autoclaved at 10 pounds' pressure for twenty minutes, to insure its sterility. It was then placed in the refrigerator at 6°C. and generally used the following day. Each sample was cultured for sterility before use, and negative results were obtained.

As the effect of thermo-sterilization was not known, the first experiments were conducted with excretions collected from maggots that had been reared aseptically. The specimens used were reared as for surgical use (Simmons, 1934 and 1935), washed from their food, and excretions collected as previously described, except for modifications to maintain sterility. This material

was cultured for sterility and used on the day it was collected. It was soon found, however, that the substance could be collected under septic conditions and then sterilized without apparent loss of potency; so the aseptic rearing was discontinued. Excretion from sterile maggots was also considered less typical of that produced by maggots in wounds.

ORGANISMS USED

A large portion of the work was done with a strain of *Staphylococcus aureus*, which is used by the U. S. Food and Drug Administration, for the testing of disinfectants and antiseptics, and has been designated by them as a standard culture which complies exactly with the specifications outlined for the normal resistance of this organism (Ruehle and Brewer, 1931).

Several strains of *Streptococcus*, both haemolytic and nonhaemolytic originally isolated from various sources such as empyema, throat infections, feces, and a knee infection were used.[2] As *Staphylococcus aureus* and *Streptococcus pyogenes* are the principal etiological agents of pyogenic infections, they received major consideration in this investigation.

Tests against *Clostridium Welchii* were conducted with a strain isolated from a fatal case of gangrene following a compound fracture of the leg. Guinea pigs inoculated with this strain were killed, with typical lesions, while antitoxin-protected controls remained alive.

Inasmuch as suppurative infections usually show a high count of *Proteus*, which are the last organisms to disappear from healing wounds, a brief test was conducted to determine the value of maggot excretions against two strains of *Proteus vulgaris*.

In addition to these typical bacteria of pyogenic infections, a test was conducted with a standard strain of *Eberthella typhi* (Ruehle and Brewer, 1931), as this is an organism much used in testing disinfectants and is sometimes important in suppurative infections.

[2] Most of the organisms used, and the information concerning them, were obtained through the courtesy of Major H. R. Livesay of the Bacteriological Department of the Army Medical School, Washington, D. C.

The bactericidal properties of the material against other and more varied forms of organisms, and its clinical applications, are to be investigated. The tests reported in this paper were conducted primarily to show the effect of maggot excretions in the disinfection of wounds.

TESTS ON STAPHYLOCOCCUS AUREUS

With excretions from contaminated maggots

The first tests with *Staphylococcus aureus* were made with cultures of various ages, an unknown number of which were removed from agar slants and suspended in physiological saline. Of the suspension, 1 cc. was placed in 9 cc. of the extract and from

TABLE 1

Effect of heat-sterilized maggot excretions on S. aureus of various ages and densities

	MINUTES' EXPOSURE						
	5	10	30	60	120	180	240
Saline control	+	+	+	+	+	+	+
Excretions	−	−*	−	−	−	−	−

+, bacterial growth; −, sterility.

* One plate showed 20 colonies after seven days' incubation.

this 1:100 and 1:1,000 dilutions were made. Identical suspensions were made in normal saline to serve as controls. These suspensions were incubated at 37°C., and plates made at various intervals, 1 cc. being used for each plate. A summary of eight complete tests is shown in table 1.

Organisms smeared on an agar plate containing 1 cc. of the material showed good growth, proving that death occurred during exposure in the tubes and that the quantity of excretions transferred to the plates would not have inhibited the growth of viable bacteria.

The results obtained indicate that maggot excretions have a strong and rapid disinfectant action on *S. aureus*.

After definitely proving the bactericidal properties of the excretions, tests were conducted on broth cultures of the standard

strain of *S. aureus*, which had been transferred to new broth every twenty-four hours for several weeks. Such cultures appeared to be more resistant to the action of the material.

A 0.1 cc. suspension of a twenty-four hour broth culture of *S. aureus*, containing approximately 50,000,000 organisms, was added to 5 cc. of the excretions and also to 5 cc. of saline. These were incubated at 37°C., and both broth and plate cultures were made at various intervals. The plate cultures of the excretions were negative in every instance, while the results obtained from two tests with the broth cultures are shown in table 2.

This test was repeated with approximately 250,000,000 organisms to 5 cc. of material. Growth was obtained at fifteen minutes, but at 60 minutes all cultures of the excretions were

TABLE 2

Effect of excretions on twenty-four-hour broth cultures of the standard strain of S. aureus

	MINUTES' EXPOSURE				
	5	15	60	120	300
Saline control	+	+	+	+	+
Excretions	+	±	−	−	−

+, heavy growth; ±, light growth; −, negative.

negative, as was the case when only 50,000,000 organisms were used. Since no cultures were made at intervals between fifteen and sixty minutes, it is possible that the less dense suspension was killed in a shorter time than the heavy one. The number of viable organisms after five- and fifteen-minute exposures was evidently small, as was indicated by absence of growth on agar plates.

One test was conducted by the agar cup-plate method. A depression was made in an agar plate thickly sown with *S. aureus*, and a small amount of the excretions placed in the cup. A clear zone of about 1 mm. surrounded the cup after twenty-four hours' incubation. Beyond this zone the substance was apparently too dilute for fatal action.

One test was conducted at 20°C. to determine if bactericidal

value was affected by temperature. Of a twenty-four hour broth culture containing approximately 50,000,000 organisms of the standard *S. aureus*, 1 cc. was subjected to 5 cc. of the excretions. Broth cultures made after fifteen minutes' exposure showed heavy growth. Another culture was not made until after two hours' exposure, at which time a very feeble growth was indicated. One test is hardly sufficient to permit definite conclusions to be drawn, but the indication is that the bactericidal potency diminishes with the temperature, a well-known phenomenon with many disinfectants.

With desiccated excreta

Of excretions from non-sterile maggots, 30 cc. were desiccated under reduced pressure at 60°C. for four hours on the day of collection and for six hours on the following day. The residue, which was still moist, was then placed in an open Petri dish and dried in an oven at 45°C. for twenty-four hours. The material was placed in the refrigerator at 6°C., allowed to remain for four days, and then diluted with 30 cc. of distilled water (the amount of liquid originally present). It was then autoclaved at 10 pounds' pressure for twenty minutes. Much of the material failed to go back into solution.

To 4.5 cc. of the supernatant fluid, placed in a test tube, 0.5 cc. of a light suspension of *S. aureus* was added. Plates made with 0.5 cc. of material at intervals of from ten minutes to two hours showed sterility in every instance, while the saline checks always showed growth. No further attempts at desiccation were made, but this test shows the possibility of obtainng the active principle in a dry powdered form.

With excretions from sterile maggots

Under the conditions involved in these tests, excretions from sterile specimens seemed to be less potent than those from non-sterile maggots. It is possible that the dilutions of the excreta from sterile maggots were greater and that the active principle maintained a constant potency. Evidence indicates the reverse, however. Several tests with this material always showed defi-

nite bactericidal effects on approximately two-week-old cultures of the standard *S. aureus*. In some instances a complete kill was obtained in ten minutes. In most cases, however, plated portions of the excretions showed some growth, but always definitely less than did the controls. Some food in which the maggots had fed was dissolved in sterile water and tested. This also showed definite bactericidal value, but not a complete kill, even at four hours.

The bactericidal action of the maggot excretions on *Staphylococcus aureus* explains in part the rapid diminution in numbers of this organism in infections under maggot therapy, as this material is constantly being discharged into the wound by the maggots.

TESTS ON STREPTOCOCCI

Haemolytic streptococci

The organisms used were haemolytic forms of *Streptococcus pyogenes*. A saline suspension of a strain isolated from a case of empyema was made from blood-agar slants and a 1:10 dilution made from this. Of each suspension 1 cc. was subjected to 9 cc. of the excretions for periods ranging from five minutes to three hours, and plates made at such intervals were always sterile. Saline checks, however, always showed growth.

Another test was conducted with the same strain of organism which had been grown in glucose broth neutralized with $NaHCO_3$. In this test a three-day-old solution of the material was used. A sample of 0.1 cc. of a twenty-four-hour broth culture, containing about 50,000,000 organisms, was exposed to 5 cc. of the excretions, and broth cultures made after five and fifteen minutes' exposure showed no growth.

A twenty-four-hour glucose-broth culture of *S. pyogenes-haemolyticus*, isolated from a throat infection, was next tried. A 0.5 cc. sample of a dense culture was subjected to 5 cc. of fresh excretions, and from this broth cultures were made at intervals of five minutes to two hours. Complete sterility was obtained in every instance. This test was repeated with a sample of material that had been kept in the refrigerator at 6°C. for three

days, and identical results were obtained. The excretions had lost some of their potency against *S. aureus* on standing, however, indicating that the streptococci were even less tolerant to its action than *Staphylococcus*.

Non-haemolytic Streptococci

Tests were conducted to determine if the viridans type of streptococcus was equally susceptible to the action of the excretions. Glucose-broth suspensions of a twenty-four-hour culture of *Streptococcus faecalis* were made from blood-agar slants, and 0.1 cc., containing approximately 50,000,000 organisms, was exposed to 5 cc. of fresh material. Cultures were made at intervals of five minutes to two hours. The five-minute cultures showed growth, but the fifteen-minute exposures were negative, as were all the rest.

This test was repeated with *Streptococcus mitior*, isolated from fluid aspirated from an infected knee, and the same results were obtained. A test with a mixed culture was conducted, in which 0.1 cc. of a twenty-four-hour glucose-broth culture of *S. faecalis*, containing about 50,000,000 organisms was used with a similar culture of *S. mitior* in 5 cc. of a three-day-old sample of excretions. The results were the same as those obtained with pure cultures. Death occurred in fifteen minutes, even though twice as many organisms and twice as much organic matter were added.

In view of these tests, the disappearance of *Streptococcus* from infected wounds under maggot therapy, as with staphylococci, can be attributed in a large measure to the action of the maggot excretions. Such action by this material in turn accounts for much of the gratifying results obtained with such therapy.

TESTS ON CLOSTRIDIUM WELCHII

Clostridium Welchii is a serious and sometimes fatal secondary invader of osteomyelitis and other infected wounds. Therefore, tests with this organism were considered of importance. The principal work was conducted with the strain previously described.

One cubic centimeter of a twenty-four-hour broth culture of

Cl. Welchii was exposed to 5 cc. of material, and 0.1 cc. of this inoculated into Robertson's chopped-meat media at intervals of from five minutes to four hours. No indication of growth from the treated organisms was apparent after twenty-four hours' incubation of the broth, while the saline checks showed heavy contamination. Subcultures were then made into litmus milk and reincubated, but no growth appeared, showing complete kill of the organism in five minutes.

A similar test was conducted with spores of a different strain of *Cl. Welchii* which had been dried in dirt. Some growth occurred even after a twenty-four-hour exposure, but death was indicated at forty-eight hours. From the viewpoint of wound disinfection, however, the resistance of *Cl. Welchii* spores is of little significance, as they are not usually encountered in such lesions.

The remarkable results obtained in the destruction of this organism with maggot excreta offers a possible explanation for the rapid clearing up of gas gangrene infections under maggot therapy, which has been observed by Baer (1931), Weil (1933), and others.

TESTS ON PROTEUS VULGARIS

Since *Proteus* is usually the last organism to disappear from infected wounds under maggot treatment, it was suspected that it might possess a higher resistance to maggot excretions than the other organisms involved. This, however, was found not to be true.

A small quantity of broth, containing at least 50,000,000 *Proteus vulgaris,* was subjected to 5 cc. of material. Plate and broth cultures made at intervals of five minutes to four hours always showed sterility. The test was repeated with identical results.

The persistence of *Proteus* can probably be attributed to its superficial position in the wound, the organism not being abundant in the deeper cavities, where the excretions of maggots naturally collect. The tests show, however, that when subjected to the action of the excretions, this organism shows slight resistance, being killed in as little as five minutes.

TESTS ON TYPHOID BACILLUS

Eberthella typhi being a standard test organism and sometimes of importance in osteomyelitis, a test was conducted[3] to determine its resistance to the excretions. The strain used was one standardized by the Food and Drug Administration of the United States Department of Agriculture for the testing of disinfectants (Ruehle and Brewer, 1931). A quantity of 0.1 cc. of a dense twenty-four-hour broth culture was subjected to 5 cc. of the material for intervals ranging from five minutes to one hour. Cultures at five minutes showed some growth, but complete sterility was obtained in fifteen minutes. Only one test was conducted, but the clear-cut results are indicative of the activity of maggot excretions against this organism.

NATURE OF THE ACTIVE PRINCIPLE

Little is known concerning the nature of the active bactericidal principle of maggot excretions. It is, however, no doubt purely nonviable. Its resistance to heat (110°C. for twenty minutes) indicates the absence of a bacteriophage or an actively bacteriolytic enzyme. It is a nonlytic agent, and apparently bears no relation to bacteriolytic elements found in tears, sputum, nasal mucus, blood serum, etc. The absence of lysis was indicated by the fact that a sample of material contaminated with a haemolytic strain of *Streptococcus pyogenes*, and from which no growth could be obtained, showed, after two days' exposure, when stained the presence of numerous dead bacteria. Duncan (1926) found that the gut content dissected from certain adult insects was devoid of any phage or lytic properties, although he was able to demonstrate bactericidal action.

The effect of dilution, beyond that which took place in collection of the material, is not yet definitely known. A sample of excreta was diluted with equal parts of 0.85 per cent saline, with no appreciable effect on its potency against *S. aureus*, whereas dilution with sterile distilled water caused a considerable drop in bactericidal action.

[3] Through the courtesy of C. M. Brewer, bacteriologist of the U. S. Food and Drug Administration, who was also kind enough to confirm certain other tests presented in this paper.

The apparent difference in activity caused by dilution with saline and with plain water might be due to some physical change.

The indications are that the excretions could be made more potent by using saline during their collection.

The durability of the principle is not known. Its potency diminishes gradually on standing in aqueous solution, but it was found to kill haemolytic streptococci in five minutes after standing for three days. Good bactericidal activity was retained for a week in dried excreta. It is interesting to note that Duncan (1926) found dried dissected gut-contents of certain insects to be as potent as fresh material after six months.

The excretions give an alkaline reaction to litmus, turning neutral paper slightly blue.

Although no tests were made, it is indicated by Duncan (1926) working along somewhat similar lines, that the active principle is tied up with minute solid particles suspended in the solution. He centrifuged and filtered through filter paper a suspension of the gut-content of a tick, *Argus persicus* Fischer, which he had previously proved to have bactericidal properties, and found that the filtrate had lost much or all of its activity. Maseritz (1934), using intestinal emulsions of surgical maggots, failed to obtain any bactericidal results. This could have been due to the fact that he used only filtered samples of emulsions of septic maggots. His failure with such material strengthens the theory put forth by Duncan.

The action of the material simulates that of phenol and similar organic disinfectants. This similarity is somewhat indicated by its poor inhibitory properties, and by the fact that it killed the organisms used in their order of resistance to such agents.

Duncan also found that the bactericidal element in the gut-content of certain insects is not of a lipoid nature, being immune to extraction by acetone, chloroform, and alcohol. He found that dried specimens of gut-content contained a large amount of insoluble material, as has been shown in the writer's experiments, and that this could be liberated by tryptic digestion and thus increase the activity of the specimen.

DISCUSSION

The foregoing experiments demonstrate that wound disinfection by surgical maggots may be due primarily to their excretions, which contain a potent bactericide. It is well known, of course, that *in vitro* tests are not always borne out *in vivo*, but the undeniable fact that remarkable destruction of bacteria occurs under the influence of maggots shows that the practical application of this substance gives positive results.

The remarkable activity displayed against both *Staphylococcus aureus* and haemolytic streptococci no doubt accounts in large part for the success of the maggot treatment of pyogenic infections, as these are the two most important etiological agents. Maggots, however, accomplish more than disinfection of the wound. Robinson (1935) has recently shown that their excretions contain allantoin, the chief end product of purine metabolism, which he has proved to be an excellent cell proliferant. Any cell proliferant would seem to benefit, however, if a bactericide were present to destroy pyogenic bacteria and make way for unobstructed action. The combination of two such factors makes for harmony, and both are present in maggot excretions. The natural coexistence of a substance that causes proliferation of soma cells with a substance that is destructive to bacterial cells is a unique phenomenon.

The good results obtained by both Baer (1931) and Weil (1933) in the treatment of gas gangrene infections by maggots is probably explained by the fatal action of the excretions on *Clostridium Welchii*. In view of these tests, and of *in vivo* tests conducted by others, it appears logical to regard the development of *Cl. tetani* in wounds as the principal remaining infection to be feared. The excretions have not yet been tried against this organism, but it is probable that at least the vegetative form would be killed. Baer (1931) however, had cases of tetanus develop in patients under maggot treatment, which indicates that such infections do not respond to the treatment so readily as gas gangrene. It is possible that the organisms were immune to the excretions, or that they were in inaccessible

cavities. The death of other organisms, elimination of necrotic tissue, and the formation of clean, healthy granulation tissue, however, tend to diminish anaerobic conditions and make less likely an infection of organisms with which maggots could not cope.

The perfection of a non-toxic, non-irritating disinfectant for internal use has not yet been realized. The apparent nature of the material here studied suggests the possibility that some form of it could be utilized in infections where maggots can not be employed.

The material is not recommended for use in the place of maggots, as they accomplish many more things than the disinfection of the wound, as has been pointed out. The investigation was made to show the rôle that maggot excretions play in wound disinfection rather than as an attempt to replace them.

From the tests described herein, it is the author's belief that intensive investigation of bactericidal substances produced by living organisms is a fertile field in which new substances might perhaps be isolated to cope with situations which do not yield to ordinary disinfectants.

SUMMARY

1. A potent bactericide has been obtained from surgical maggots of the species *Lucilia sericata*, and the technic for its collection is described.

2. Bactericidal tests with this substance were conducted with seven species of bacteria of etiological importance in pyogenic infections. The results showed that five- to ten-minute exposures were usually sufficient to give 100 per cent kill of dense saline and broth suspensions of the organisms.

3. The addition of organic material apparently has less effect on the potency of this material than on that of ordinary disinfectants.

4. The active principle is of a nonviable nature, and is not destroyed by autoclaving for twenty minutes at 10 pounds' pressure.

5. No indication of lysis could be demonstrated, and the

thermostability and other reactions of this substance rule out the possibility of a bacteriophage as the active principle.

6. The material was desiccated, and in this dry condition it apparently maintains its potency over a longer period than when in aqueous solution.

7. The remarkable bactericidal potency of the excretions against *Staphylococcus aureus*, haemolytic streptococci, and *Clostridium Welchii* accounts in part for the gratifying results obtained in such infections under maggot therapy.

8. The investigation reveals a field with potentialities of producing other new and useful disinfectants from living organisms.

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