A PHARMACEUTICAL STUDY OF LUCILIA SERICATA

By

HENRY STANLEY KROMRAJ

A Thesis Submitted for the Degree of

BACHELOR OF SCIENCE

(Pharmacy)

UNIVERSITY OF WISCONSIN

1942

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I. INTRODUCTION

Maggots of certain species of the flesh flies have been used successfully in the treatment of purulent deep seated wounds. Baer[1] in 1931 proposed with success the new method of using the maggots of the flesh fly Lucilia sericata as the therapeutic agent. The success of his method led to the establishment of raising sterile maggots to be used in maggot therapy.

The use of the maggots themselves was somewhat repulsive to the medical man and the patient. To get away from the use of the live maggots Livingston and Prince[2] using a saline extract of the whole maggots, successful treated 88% of 200 chronic osteomyelitis cases. They claimed that the maggot extract contained a sulphydryl, natural allantoin, calcium, cysteine, glutathione, and in addition an embryonic growth stimulating substances. The maggot extract they concluded was the therapeutic agent and not the maggots themselves.

Robinson,[3,4] using products found in the secretion of the live maggots successfully treated purulent wounds. The products were allantoin, urea, and ammonium acid carbonate. These compounds in Robinson's work were applied separately and

II. EXPERIMENTAL

Maggots of the type which are used in maggot therapy were obtained from Hynson, Westcott and Dunning, Inc., The maggots were raised to maturity by being fed beef. All beef referred to in this paper is boneless round. After maturity the maggots were allowed to pupate and the pupae placed in cages where they developed into flies. The cages were constructed of a size to support from three to four hundred flies each. A suitable opening for feeding the flies was arranged for.

The flies obtained were classified zoologically as Lucilia sericata,[6] and were used as breeders from which the maggots for the experiments were obtained. No difficulty in raising the flies in the laboratory existed if the room temperature was 20° C. or above. The food for the successful raising of these insects was the Baer Mixture,* plenty of water, a source of sugar, either granular or cubed.

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* Baer Mixture
70 cc. of water
30 cc. of honey
one half cake of yeast
one egg
Mix the ingredients together and place a little amount on a pad of absorbent cotton into the cage twice a week.

in the form of ointments, solutions, and as a dusting powder. The proof of the presence of these compounds in the secretion was carried out by chemical tests.[5]

Since the extract used by Livingston and Prince was successful, it was concluded that by extracting the maggots just before the prepupal stage with selective organic solvents and using the different extratives on wounds, the extract most effective might be determined. Thus, the use of live maggots in the treatment of disease tissue might be eliminated. After finding a successful extract the principle constituents could be isolated.

The object of the thesis deals with the development of the maggots for laboratory work, and the preparation of various extracts using different solvents. A description of the material extracted and some preliminary work on the constituents is included.

In one observation when the Baer Mixture was omitted and the beef substituted, egg production was limited.

The life cycle, egg, maggot, pupa, and adult was shortened measurably when the temperature of the laboratory was 29° C. or slightly above.

A. Raising the Maggots

Eight days after emergence and properly fed the flies are ready to lay eggs. A piece of beef was placed in the cage and left over night on which the flies deposited their eggs. The meat which contained the eggs was removed from the cage and placed in a six hundred or an eight hundred cc. beaker containing more of the beef. The beaker was covered with a double thickness of cheese cloth and placed in a dark, warm place. On the third day the beaker holding the maggots was set into a larger container, usually a two liter beaker, and the cheese cloth removed and placed over the larger. A rubber band will suffice to hold the cloth securely in place. The maggots leave the beef and climb from the small beaker, and fall into the large one from which they are collected. The maggots are then washed to remove any foreign matter.

Another method of collecting the maggots after three days of feeding upon the beef is to place the contents of the small beaker upon an eight mesh screen. By placing a light over the contents it will cause the maggots to crawl through the holes in the screen and fall into a receptacle suitably

placed to collect them. The maggots to be used in the experiments were collected just before reaching the prepupal stage.

B. Preliminary Extraction

The maggots obtained in this manner were subjected to extractive exhaustion in a modified Soxhlet apparatus with different organic solvents. Some difficulties occurred when petroleum ether (Skelly B.) was used. The petroleum ether would not flow through the maggots. The petroleum ether coming in contact with the maggots formed a gelatinous mass which slowed the flow of the petroleum ether into the receiving flask. Ir was later found to be caused by the moisture and precipitated proteins in the maggots. When mixed with pure sand some progress of extraction was observed, but only to a small extent.

This slow extraction with petroleum ether was carried on until the gelatinous condition disappeared and the succeeding portions of the solvent flowed through the mixture in the apparatus without causing an uneven flow of the continuous extraction. It was also observed that glass beads mixed with the maggots have the same effect as pure sand.

Petroleum Ether (Skelly B.)

The extract obtained in the receiving flask consisted of three separate portions. A fraction soluble in the Petroleum ether which upon evaporation of the solvent consisted of an oily yellowish liquid having an odor of decomposed flesh. The petroleum ether soluble fraction of 19° C. formed

some crystals suggesting some free fatty acids. An insoluble portion which was recovered by filtering, consisted of a light brown amorphous solid. The third fraction was a liquid insoluble material heavier than the petroleum ether and upon drying left a dark brown resinous residue.

The solid insoluble fraction found in the solvent was insoluble in water, concentrated HCl, ether, and alcohol. It was readily soluble in warm 10% NaOH and in concentrated sulfuric acid.

Ether Extract

Upon successive continuous extraction of the same maggots in the modified Soxhlet apparatus with ether, more of the insoluble solid material obtained in the petroleum ether was deposted in the receiving flask. Upon the removal of this material and the recovery of the ether a yellow oily liquid was obtained. The oily liquid deposited crystals when subjected to a temperature of 19° C. and appeared similar to those from the petroleum ether extract. The odor of the oily liquid was not characteristic of decayed flesh, but more ammoniacal.

Acetone Extract

Acetone was the next solvent used on the maggots in the extraction apparatus, and after exhaustion by continuous extraction the acetone was removed. Upon complete evaporation the residue consisted of an oily, brownish, resinous like mass. The odor is best described as that of "crisp" bacon.

Chloroform Extract

After complete exhaustion by continuous extraction with chloroform the residue left after evaporation contained a small amount of oily material. This residue could have been similar to that obtained from the acetone extract.

Alcohol Extract

The maggots from the previous extractions were subjected to further continuous extraction with alcohol. After exhaustion the alcohol was removed and the residue remaining was a yellowish gummy powder. During the process of evaporation of the alcohol and especially when the solution became concentrated the material had the appearance of flocculent crystals.

Total Extractive

One hundred and thirty-five grams of the maggots were collected and extracted with the above organic solvents. The amount of material obtained in each extraction was determined. The results are tabulated below.

Solvent Used	Weight of the Extract	Per cent
Petroleum ether (Skelly B.)	Oily soluble residue 2.86 Gms.	2.11
	Solid insoluble fraction 12.2 Gms.	9.03
	Liquid insoluble fraction 16.34 Gms.	12.1
Ether	Soluble portion 2.56 Gms.	1.89
	Insoluble portion 9.32 Gms.	6.9
Acetone	Residue 4.175 Gms.	3.09
Chloroform	Residue .568 Gms.	.42
Alcohol	Residue 2.221 Gms.	1.64
Residue left	Hulls 23.42 Gms.	17.35
Loss	61.336 Gms.	45.47

Moisture Determination

A moisture determination was made using a Deane-Stark apparatus. Two samples one of 5.05 Gms. and one of 5.3 Gms. were used. The moisture content of the two samples averaged 60%. Some of the loss in the total extractive table may be due to the moisture in the maggots.

C. Examination of the Oily Material of the Preliminary Extraction

The oily fractions from several small petroleum ether, ether, and acetone extractions were combined. A partial examination was carried out on this oily material.

To 9.5 grams of the oily material 80 cc. of alcoholic Koh was added and this mixture was refluxed for five hours.[7] The mixture was cooled and shaken with five 20 cc. portions of ether in a separatory funnel. The combined ether portions upon evaporation left a residue which gave a positive Salkowski and Liebermann-Burchard[8] tests for sterols.

The ether residue was next dissolved in absolute alcohol and enough distilled water was added to dilute the alcohol. In the diluted solution crystals formed which were removed by filteration. The crystals were pearly white in appearance, recrystallization from absolute alcohol melted at 142-3° C. This observed melting point would indicate that the crystals were cholesterol.

The mother liquor after the removal of the crystals was evaporated and a soapy albuminous residue remained.

That portion which remained after extracting with ether was neutralized with 10% HCl and shaken with five 20 cc. portions of ether. Upon evaporation of the ethereal solution a pale yellow liquid remained having a pronounced butyric odor. At 30° C. it was liquid and at 24° C. it crystallized. The characteristic odor and the behavior of the liquid at the

above temperature would indicate the presence of the lower fatty acids. No further examination was carried out at this time.

A test for the presence of glycerin in the residue after the removal of the saponified material was made. A portion of the residue was heated with potassium acid sulfate and an acrolein odor was detected. If glycerin is present then it becomes apparent that some of the fatty acids must be combined as glyceryl esters.

D. Preparing Maggots for Succeeding Extraction

Maggots were collected from the beef as previously described for this extraction. In the preliminary extraction they had turned brown and were considered undesirable for the purpose intended. If they could be kept in their natural condition throughout the extraction the extracts obtained would be more nearly the same as in the maggots at the time of collection. The moisture in the maggots was thought to be responsible for the change.

The first suggestion for the removal of the water was that of slow evaporation of frozen maggots. The maggots were placed in a vacuum container in which methyl alcohol cooled by CO_2 ice acted as the cooling agent. The temperature was lowered to -10° C. and the maggots were kept at this temperature for five hours. After five hours the frozen maggots were placed in a dessicator containing calcium chloride

and were allowed to slowly reach room temperature. As the temperature approached this point they revived and showed signs of activity.

A second portion of maggots were cooled to a temperature of -30° C. and these did not revive, however, they turned brown upon dehydration.

In Acetone

For another experiment maggots were collected previous to the prepupal stage. These were washed several times in distilled water. The water was removed by decantation and the last washing was removed by suction with a Buchner funnel. The suction took off all the external water. The maggots then were placed in a container and covered with acetone. They turned brown and hard after twelve hours. The acetone was recovered and left a pale yellow liquid with a few crystals.

In Alcohol

Another portion of maggots were washed as above and placed in absolute alcohol. After twenty-four hours no change in their appearance could be seen. The alcohol was removed from the maggots after they had been in the alcohol thirty-six hours. These maggots were used in the succeeding extraction.

E. Extraction of the Alcohol Dehydrated Maggots

One hundred and fifty grams of alcohol dehydrated maggots were placed in a modified Soxhlet extractor. Succeeding extraction with two hundred cc. portions of petroleum ether, acetone, and alchol was carried out on this group of maggots. These solvents were selected for they gave the greatest amount of extractive in the preliminary work.

Petroleum Ether

With petroleum ether there was no retardation of the solvent through the maggots as had occurred in the preliminary extraction. After exhaustion with this solvent the residue consisted of three parts. A soluble portion upon evaporation of the solvent was a light, oily, yellowish liquid. The insoluble solid portion when separated by filteration and dried was a solid light brown mass. An insoluble liquid fraction was separated with a separatory funnel. It consisted of a reddish brown liquid and upon drying turned into a brown viscid material.

Acetone

Acetone was the next solvent used on the maggots in the Soxhlet extractor. This solvent carried into the receiving flask a soluble material and an insoluble liquid. The former on separation from the insoluble liquid and on evaporation of the acetone left a heavy viscid, dark, brown residue. While the acetone was evaporating crystals appeared to be forming.

The residue was soluble in water. Upon addition of acetone a precipitate formed which was separated by means of filtering with suction. A test for nitrogen was positive.[9] After two crystallizations the material melted at 231° C. with decomposition.

The insoluble liquid portion was an oily yellowish liquid which floated on the acetone in the receiving flask.

Alcohol Extractive

The extraction was completed with the use of alcohol. The residue obtained on removal of the alcohol was a brown viscid mass, and was soluble in water. It did not resemble the residue that was obtained in the preliminary extraction. When acetone was added to a water solution of the residue a precipitate formed. The precipitate separated by suction with a Buchner funnel was a yellowish, amorphous mass. It had the same appearance as the precipitate of the acetone soluble residue.

III. SUMMARY

1. The study showed that Lucilia sericata flies can be kept in a laboratory without controlled conditions.
2. Maggots for extraction are obtained in collectible quantities.
3. The favorable solvents found were petroleum ether (Skelly B.), acetone, and alcohol.
4. The maggots were best prepared for extraction by dehydrating with alcohol.
5. The extracts appeared to be suitable for incorporation into ointments.

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Approved by A. H. Uhl
Professor of Pharm. Chem.

Date August 28, 1942