OBSERVATIONS ON THE NUTRITION OF MAGGOTS OF AUSTRALIAN BLOW-FLIES

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INTRODUCTION.

One phase of the blow-fly research of the Division of Economic Entomology is an investigation of the food and conditions of life of the maggot on the living sheep. This work has included both studies of the development of the larvae on the living sheep and experiments in vitro on the nutrition and bionomics of the larvae. It is with the in vitro experiments that this paper chiefly deals.

R. P. Hobson in three recent papers (Hobson, 1931 a, 1931 b, 1932 a) has demonstrated that the excreta of third instar larvae of Lucilia sericata Mg. contain proteolytic enzymes. Strong tryptic activity was noted, but a peptic enzyme appeared to be absent. He found the tryptic enzyme in sterile larvae as well as those reared in association with bacteria.

It has recently been shown by Miss M. Fuller in this laboratory that the fly which causes most trouble to sheep in Australia is L. cuprina Wied. (Fuller, 1932), and that L. sericata appears to be of less serious economic importance. It therefore appeared desirable that the observations of Hobson should be confirmed and extended using L. cuprina. Experiments have also been made with L. sericata Mg. and Chrysomyia rufifacies Macq.

Prior to the work of Hobson, Wollman as early as 1911 pointed out that proteolytic enzymes were present in the excreta of sterile larvae (Wollman, 1911). Literature dealing with the nutrition of blow-fly larvae has been well summarised by Uvarov (1928) and more recently by Hobson (1931, 1932) and is therefore not listed here. Various workers have succeeded in rearing sterile maggots (Frew, 1928; Baer, 1931; Hobson, 1932; Causey, 1932), and a study of these has received an impetus by the work of Baer on osteomyelitis. So far as we know, the work reported here is the first specifically designed to elucidate the relation of the larvae to the living sheep. It has consisted of:

1. Observations on the growth of larvae on various media, including wool.
2. Rearing larvae of L. cuprina, and Ch. rufifacies aseptically.
3. Study of the enzymes secreted by the larvae of L. cuprina, L. sericata and Ch. rufifacies.
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(4) Chemical examination of the fleece for the purpose of determining the presence of soluble protein.

We are indebted to Dr I. M. Mackerras for his active interest, advice and criticism, and to Mr C. R. Mulhearn for material and many helpful suggestions.

OBSERVATIONS ON THE GROWTH OF MAGGOTS.

(1) Growth of maggots in carrion. It is well known that blow-fly maggots grow quickly in fresh carrion with rapid liquefaction of the medium. When protected from desiccation, maggots will reduce a piece of fresh meat, either muscle or liver, to a black semifluid mass in a few days, the time needed decreasing with increasing temperature. That this liquefaction is due largely to the maggots is seen by comparing the appearances of equal masses of liver exposed to the same external conditions for equal periods, when one is allowed to putrefy without maggots and the other in their presence. When maggots are present in excess, the liver first becomes reduced to a spongy framework enclosing a blackish fluid and finally, if only assimilable tissue is present, nothing will be left but some blackish slime. Under these conditions the reaction rapidly becomes strongly alkaline and ammonia is given off. This, according to Hobson (1932b), plays an important part in the further disintegration of the medium by activating the tryptic enzyme of the larval excreta and by changing the colloidal properties of the proteins.

Without maggots, the liver goes through a stage of softening and then gradually dries up. The reaction becomes strongly acid and remains so for several days and finally becomes alkaline.

The action of the maggots on the meat is manifold:

(i) A mechanical fragmentation of the meat by their jaws and muscular movements. We have found particles of irregular shape measuring up to $75 \times 50 \mu$ in the crops of L. cuprina maggots feeding on liver. It has often been stated that maggots can only absorb liquid food. It would be more correct to say that their food must be in a finely divided state.

(ii) A liquefaction by digestive enzymes; this is dealt with in more detail below.

(iii) An indirect action, aiding the growth of proteolytic aerobes by disseminating the organisms throughout the medium, by raising the temperature and by neutralising the acidity of the medium.

(2) Growth of maggots on the living sheep. On the sheep growth is, if anything, more rapid than on carrion, but it is not clear on what elements, if any, the larvae feed immediately on hatching and before the skin reaction begins. Free moisture is, however, essential. Once the maggots begin to work over its surface, the skin becomes intensely irritated, either mechanically by the jaws of the maggots, or by the secretions of the larvae, or by the action of the bacteria they disseminate, most

1 When two equal masses of meat are putrefying, one with and the other without maggots, the temperature of the former is higher than that of the latter. With large numbers of third instar maggots an increase of 3°C. over the control has been noted.
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probably by all these factors acting together. A serous exudate then appears. This may be so copious that it can be collected in a test-tube. One sample thus collected was found to contain a coagulable protein, probably albumen, and peptones. Stained smears examined microscopically showed a few leucocytes, occasional red cells and very numerous bacteria. This exudate forms a suitable food for larval development and is added to a little later by fresh blood from the acutely inflamed and broken skin surface. Fresh blood may be seen in the gut of the maggots. The wool fibres become discoloured, at first yellow from the exuding serum, and they gradually become blackened from the decomposing blood; a characteristic and disagreeable odour is present.

Occasionally small numbers of maggots may be found developing away from the skin surface in faeces-soiled wool and experiments have been carried out to test the suitability of raw wool in vitro as a medium for larval growth.

(3) Growth of maggots in raw wool in vitro. Various samples of wool from the crutch and body have been tested. The method adopted was to saturate the sample of wool with sterile water and place it on sterile damp sand in a glass jar. Fifty newly hatched maggots were then placed on the wet wool and the jar kept at 22° C. No development occurred in clean body wool, but complete development occurred in some samples of crutch wool and in samples of body wool taken from a previously struck area. The sheep, from which the crutch wool was taken, all showed some irritation of the skin, so that there was probably more than merely faecal material available as larval food. Bull (1931) has demonstrated the presence of dermatitis in the breech folds, even of young lambs, and some serous exudation would certainly be present in these areas. The two samples of body wool from previously struck areas showed a narrow band of hard yellow material, referred to below as a gummy crust. This became soft and sticky when wet and on it maggots developed slowly at first, but attained full size in a week, pupated and emerged as normal flies. This gummy crust was obviously the dry exudate from the previous strike. Being limited in amount one would expect only a limited number of maggots to develop on it and this was found to occur. Less than half the number of maggots originally added developed in each sample, and after they had developed the samples were rewetted and more newly hatched maggots added. None of these developed, however, showing that the first batch had used up the available food. At the end of the larval development the wool had acquired the odour characteristic of strike, but no blackening of the fibres occurred.

It has been noted by other workers, and lately by Dr F. G. Holdaway and Mr C. R. Mulhearn in this laboratory, that weather stain in the wool is sometimes associated with strike. Tests were therefore made with two samples of green-stained wool. In one case the stain had developed spontaneously in the field and in the other clean body wool had been wetted and inoculated with B. pyocyaneus and incubated for 5 days at 31° C., by which time a bright green staining of the fibres had been produced. The same technique was followed as in the other experiments. No development of the maggots occurred, although they survived for 2 to 3 days in the moist wool.
These experiments are set out in tabular form below:

Table 1.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Species</th>
<th>Sheep No.</th>
<th>Weight of wool (gm.)</th>
<th>Condition of wool</th>
<th>Stage reached by maggots</th>
<th>No. of flies emerged</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>L. cuprina</em></td>
<td>Ewe</td>
<td>5</td>
<td>Body wool showing a gummy crust</td>
<td>3rd instar</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>&quot;</td>
<td>10</td>
<td>Clean body wool</td>
<td>&quot;</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>&quot;</td>
<td>5</td>
<td>Body wool showing artificial green stain</td>
<td>Nil</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>&quot;</td>
<td>5</td>
<td>Body wool showing natural green stain</td>
<td>&quot;</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>&quot;</td>
<td>&quot;</td>
<td>10</td>
<td>Crutch wool, soiled with urine from area of skin showing much irritation</td>
<td>3rd instar</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>&quot;</td>
<td>8</td>
<td>Crutch wool much soiled with faeces</td>
<td>&quot;</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
<td>&quot;</td>
<td>7</td>
<td>Crutch wool slightly soiled with faeces</td>
<td>&quot;</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>&quot;</td>
<td>&quot;</td>
<td>25</td>
<td>Crutch wool soiled with faeces</td>
<td>&quot;</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>&quot;</td>
<td>&quot;</td>
<td>51</td>
<td>Crutch wool soiled with faeces</td>
<td>&quot;</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>&quot;</td>
<td>&quot;</td>
<td>47</td>
<td>Crutch wool slightly soiled with faeces</td>
<td>&quot;</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>&quot;</td>
<td>&quot;</td>
<td>47</td>
<td>Crutch wool soiled with faeces</td>
<td>&quot;</td>
<td>13</td>
</tr>
<tr>
<td>12</td>
<td><em>Ch. rufifacies</em></td>
<td>48</td>
<td>&quot;</td>
<td>Nil</td>
<td>3rd instar</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td><em>L. sericata</em></td>
<td>48</td>
<td>&quot;</td>
<td>Nil</td>
<td>3rd instar</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>&quot;</td>
<td>45</td>
<td>&quot;</td>
<td>Crutch wool slightly soiled with faeces</td>
<td>&quot;</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td><em>Ch. rufifacies</em></td>
<td>46</td>
<td>&quot;</td>
<td>Crutch wool soiled with faeces</td>
<td>&quot;</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td><em>Caliphora stygia</em></td>
<td>46</td>
<td>&quot;</td>
<td>Crutch wool soiled with faeces</td>
<td>&quot;</td>
<td>2</td>
</tr>
</tbody>
</table>

(4) Growth of maggots in sheep dung. Maggots will survive in fresh sheep dung, but growth is very slow. In one experiment with *L. sericata* the maggots grew slowly and after 24 weeks one very undersized maggot pupated, but a normal fly was not obtained. We have insufficient experimental evidence to generalise, but it seems unlikely that this species can develop normally on sheep dung alone.

Faecal soiling is, however, important in attracting flies to the crutch and in providing initial food for the maggots; the exudate from some previous irritation plays the same part in body strike and probably also in crutch strike as well. The wool fibres themselves do not appear to be attacked by the maggots. Hobson (1931 b) could not demonstrate a keratinase in the excreta of *L. sericata* maggots. Some experiments were carried out to determine how far maggots could develop on the substances obtained by hydrolysing pure keratin.

(5) Keratin derivatives as food for maggots. Raw wool was extracted with ether, washed with water and the keratin obtained was digested with *5N* acid until the
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Fibrous structure was destroyed. The digest was then neutralised and filtered. The filtrate was dialysed until none of the acid used for hydrolysis could be detected within the membrane, it was then concentrated in a water bath. The fraction consisted chiefly of peptones and proteoses. The residue obtained from a similar acid digestion was filtered and washed continuously until free from acid.

Small portions of these substances, also of wool wax obtained by ether extraction, were placed on moist filter paper in petri dishes, either alone or in mixtures with one another. Small numbers of newly hatched maggots of *L. sericata* and *Ch. rufifacies* were added, the petri dishes were kept at 22° C. and the filter papers moistened daily. Controls with small pieces of liver were also set up.

The filtrate from acid hydrolysis containing proteoses and peptones was the most favourable for development, though growth was very slow. In this, and in mixtures containing it, both species reached second instar and a few *L. sericata* reached third instar and one even pupated. Development was, however, very imperfect and no fly emerged. No growth occurred on pure wool wax. The controls on liver developed normally. It was noticed that the maggots lived longer when the medium was slightly alkaline and died without growing at all when it was slightly acid.

These experiments indicate that maggots can develop to a certain extent on a mixture of proteoses, peptones and polypeptids, even though these are derived originally from keratin, which is comparatively stable and resistant to hydrolysis by larval enzymes (Hobson, 1931 b).

**REARING STERILE MAGGOTS.**

Egg masses were obtained by placing fresh liver in petri dishes in the cages containing stock flies. The eggs were allowed to incubate until almost ready to hatch, for at this stage, as noted by Hobson, they are more resistant to the toxic action of the disinfectant. They were then placed in an aqueous solution containing 1 per cent. crystalline sodium sulphide, which, by dissolving the cement substance which binds the eggs together, allows their ready separation. The eggs were then washed thoroughly with distilled water and placed in the sterilising fluid. Various strengths of HgCl₂ were tried and the best results obtained with 0.4 per cent. aqueous solution for 10 minutes. Only those experiments were successful in which very small numbers of eggs were used. When attempts were made to sterilise eggs on a large scale the resulting cultures were always contaminated. This was probably due to the eggs clumping together and thus preventing perfect contact with the disinfectant.

After treatment with mercuric chloride, the eggs were poured into a filter funnel containing a little cotton wool (the whole having been previously autoclaved). They were then washed thoroughly with sterile water and transferred aseptically to sterile nutrient media. In the experiments with *L. cuprina* a small cube of liver was placed in a fairly wide-mouthed bottle, some baker’s yeast and nutrient agar added, and the whole autoclaved for 20 min. at 15 lb. pressure. The mouth of the bottle was

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1 *L. cuprina* was not available when these experiments were carried out.
firmly plugged with cotton wool. Cultures were kept at 31° C. and examined at intervals by making smears and subcultures from the agar and smears from the gut of the maggots.

Maggots in sterile culture grew more slowly than in the presence of bacteria, but eventually reached full size, pupated and emerged as normal adults. The slowness of growth is probably not to be explained by the absence of bacteria but, as Wollman suggests, by the relative indigestibility of the coagulated (autoclaved) protein and by reduction of vitamine content (Hobson, 1932c). Some hydrolysis would probably occur, however, in the production of the medium, which was moist and contained a well-defined cube of liver. As the maggots grew, they produced a considerable amount of disintegration of the liver with the production of a greyish slimy fluid which they carried all over the tube.

In the experiments with Ch. rufifacies the same medium was used, but nutrient broth was substituted for agar in order to bring the medium to the more fluid consistency preferred by this species. On this medium single isolated maggots were reared in sterile culture on three occasions, and produced normal puparia and flies. At the beginning of the experiment, the medium consisted of a small cube of liver partly immersed in clear broth. Disintegration of the liver was more rapid and complete than was the case with L. cuprina, the whole medium at the end of the growth being reduced to a thin, almost homogeneous, pasty, brown mass. Neither in the culture of L. cuprina nor Ch. rufifacies was there any odour of putrefaction.

Thus, larvae both of L. cuprina and Ch. rufifacies can liquefy and absorb their food without the aid of bacteria. Moreover, Ch. rufifacies can mature normally without a diet of living larvae, and the predatory habit so commonly observed in the field and stressed by Patton (1922) is not essential for normal development.

STUDY OF THE ENZYMES.

The object of the investigation was to determine the presence of proteolytic enzymes in the excreta of the larvae of L. cuprina in different stages of their growth and also in the excreta of third instar larvae of L. sericata and Ch. rufifacies.

Eggs of L. cuprina were collected by placing a small piece of liver in the pure cultures of the flies. When unfed larvae were required, the eggs were removed from the liver, washed and allowed to hatch upon damp filter paper. Otherwise the eggs were allowed to hatch on the liver, on which the larvae of the fly under examination fed until they had reached the desired stage in their growth. They were then removed and separated from all debris by washing in warm water and were placed in a thistle funnel which was plugged and covered with muslin to prevent their escape. At intervals about 5 c.c. of distilled water was poured over the larvae and was collected as it drained away. This treatment was continued for periods varying from 5 to 24 hours, depending upon the size and number of the maggots used.

The liquid collected varied in appearance from clear opalescent to dark brown and was always strongly alkaline. Aliquot portions of this were then allowed to act on 1 per cent. gelatine solution at 31° C. for periods up to three days, the reaction
being controlled by means of buffer solutions. Activity at pH 8.0, obtained by means of a phosphate buffer, is due to tryptase and that at pH 2.4 obtained with a phthalate buffer is due to peptase. After a period of incubation, the mixture was brought to neutrality and neutral formaldehyde was added. Any increase in acidity recorded is a measure of the digestion which has occurred and therefore indicates the presence of proteolytic enzymes. A portion of the liquid was boiled to inactivate the enzymes and control experiments under identical conditions were performed in each set of experiments. The corrections indicated by these controls have been applied before interpreting the results.

In preliminary experiments thymol was added to inhibit bacterial activity. Toluene, however, was found to be definitely bactericidal. It is without effect on enzymes, and was consequently used in the experiments reported in this paper.

Conclusions based upon the results of twenty experiments are set out in the appended table. For the purpose of comparison the following signs have been used: ++ strong, + definite, ± very slight activity.

<table>
<thead>
<tr>
<th>Species</th>
<th>Stage</th>
<th>Peptic</th>
<th>Tryptic</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. cuprina</td>
<td>1st instar unfed</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>L. cuprina</td>
<td>1st instar fed</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>L. cuprina</td>
<td>2nd instar</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L. cuprina</td>
<td>3rd instar</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>L. cuprina</td>
<td>Early prepupal</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>L. cuprina</td>
<td>Late prepupal</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>L. cuprina</td>
<td>3rd instar</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>L. sericata</td>
<td>3rd instar</td>
<td>±</td>
<td>++</td>
</tr>
</tbody>
</table>

The excreta of sterile larvae was not examined, and so some weak proteolytic activity recorded might be due to enzymes collected from the bacteria present in the maggots. It may be noted that we have isolated proteolytic organisms from the excreta of maggots.

Strong peptic activity was not noted, but peptase was definitely present in some cases. Though the methods used were satisfactory for the detection of tryptase, they were less suitable for estimating peptase, so a further series of experiments is planned to determine more definitely the presence of peptase in the younger larval stages.

It is obvious from these results that newly hatched maggots of *L. cuprina* secrete an enzyme which is capable of digesting protein. Apparently tryptase is more abundant than peptase and would be far more active under the normal conditions of larval life, i.e. upon carrion, which quickly becomes alkaline in reaction when crowded with larvae, as it always is in nature.

**CHEMICAL OBSERVATIONS ON THE FLEECE.**

While examining fresh wool samples in an attempt to obtain some correlation between strike in sheep and different fractions of the wool, a technique has been developed which allows different wools to be compared and which gives some idea of their constituents. Although the technique has not yet been perfected nor used to
examine a large series of wools, it seems desirable to publish an account of the work at this stage and to indicate some results which have been obtained in the preliminary experiments.

The wool sample under examination was steeped in ten times its weight of water for 24 hours, thymol being added to inhibit bacterial activity. The contained liquid was then expressed and filtered and two portions of 20 ml. were taken. One of these was titrated in water to phenolphthalein with \( \text{N/100} \) potassium hydroxide solution before and after the addition of formaldehyde, as in the Sorensen titration, and the other was titrated in ten times its volume of alcohol, as in the Foreman titration. Both the formaldehyde titration and that in alcohol should give closely corresponding figures, as both depend on the inactivation of the amino groups of the proteins present.

A table of some of the results obtained is set out below. All the figures represent ml. of \( \text{N/100} \) KOH needed to neutralise the solutions to phenolphthalein.

**Table III.**

<table>
<thead>
<tr>
<th>Sheep No.</th>
<th>Description of wool</th>
<th>Portion of staple</th>
<th>( \text{Ml. N/100 KOH used in titration} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>In water alone</td>
</tr>
<tr>
<td>15</td>
<td>From unstruck area</td>
<td>Tip</td>
<td>3.4</td>
</tr>
<tr>
<td>15</td>
<td>From unstruck area</td>
<td>Base</td>
<td>1.2</td>
</tr>
<tr>
<td>40</td>
<td>From unstruck area</td>
<td>Tip</td>
<td>1.3</td>
</tr>
<tr>
<td>40</td>
<td>From unstruck area</td>
<td>Base</td>
<td>3.4</td>
</tr>
<tr>
<td>40</td>
<td>From unstruck area</td>
<td>Whole</td>
<td>0.9</td>
</tr>
<tr>
<td>40</td>
<td>From struck area</td>
<td>Whole</td>
<td>4.5</td>
</tr>
<tr>
<td>43</td>
<td>From unstruck area</td>
<td>Whole</td>
<td>3.2</td>
</tr>
<tr>
<td>43</td>
<td>From struck area</td>
<td>Whole</td>
<td>3.2</td>
</tr>
<tr>
<td>43</td>
<td>From struck area</td>
<td>Whole</td>
<td>0.0</td>
</tr>
<tr>
<td>43</td>
<td>From struck area</td>
<td>Whole</td>
<td>0.5</td>
</tr>
</tbody>
</table>

The extracts of most wools examined, whether struck or unstruck, were almost neutral in reaction to phenolphthalein when titrated in aqueous solution. However, when the basal and distal portions of the staple of unstruck wool were titrated separately, the distal portion was found to be more acid than the base. This is in general agreement with the work of Lifschutz (1924), who found the wax of the tip to be more acid than that of the base, and of Barritt and King (1929), who found that the wool fibre is partially decomposed by light and that some of its sulphur is converted to sulphur dioxide.

When the extracts were titrated in water after adding formaldehyde, there was little increase in acidity with unstruck wool, but a large increase in acidity was noted at this stage when struck wool was titrated. This would be due partly to an increase in the protein content of the sample and partly to the destruction of any ammonia present.

The figures obtained by titration in water when formaldehyde is added, and in the alcoholic solution, are of interest. In every case the alcoholic solution is more acid than the formaldehyde treated solution. If this acidity were due simply to the
destruction or inactivation of amino groups, the figures obtained by both methods should be almost identical, as is shown by the figures of Sørensen and Katschioni-Walther (1928). The excess acidity in alcohol is probably due to higher fatty acids, which being insoluble are not titrated in water, but which may be dissolved and titrated in alcoholic solution. Little increase in acidity occurs when formaldehyde is added to the neutralised extract in alcohol.

These results throw some little light upon changes occurring during strike. It has been shown that maggots normally excrete ammonia and one would, therefore, expect that the struck area would become strongly alkaline. However, extracts made by the methods described above varied little from neutrality to phenolphthalein. The excess acidity in alcohol, suggested as being due to higher acids insoluble in water, increases greatly during strike and the reason for the continued neutrality is probably that the ammonia liberated by the larvae is combined with these acids. There is also an increase of soluble protein content during strike and this would supply nutrient material for the larvae. By using the precipitin reaction with specific anti-sheep serum, Dr I. M. Mackerras has also demonstrated an increase in soluble sheep protein in the wool of struck areas.

SUMMARY AND DISCUSSION.

1. Larvae of *L. cuprina* and *Ch. rufifacies* are capable of liquefying and digesting protein media without the intervention of bacteria. Both species and *L. sericata* secrete trypic and peptic enzymes and at least *L. cuprina* does so from the moment of hatching. Trypsin is more abundant than peptase. Predatory activity, though a normal habit of *Ch. rufifacies*, is not necessary for any of these species and did not occur in the masses of *Lucilia* larvae used for extraction of the enzymes, although they were kept for periods up to 24 hours without food.

2. Partial development of larvae occurred in sheep dung, faeces-stained wool and in the products of keratin hydrolysis. Complete development took place in wool containing a "gummy crust" of dried exudate and in some samples of faeces-stained wool. Some of the samples of faeces-stained wool and those containing a "crust" have been demonstrated serologically by Dr I. M. Mackerras to have an increased content of soluble sheep protein as compared with normal wool. A marked increase of soluble protein has been demonstrated in struck wool both chemically and serologically.

3. Moisture, warmth, shelter and aeration are essential physical conditions for larval development. In addition, an alkaline reaction is relatively favourable and an acid reaction relatively unfavourable.

4. There are normally two stages in the development of a primary strike, the first stage being from hatching up to the time the larvae attack the skin, the outer layer or epidermis of which is approximately 36 μ thick (Whitnall, 1931). During this stage they must feed, if at all, on materials already present. The second stage is from the commencement of an actual skin lesion up to full development of the maggots. During this stage there is a more or less copious serous exudation, which
has been shown to be an adequate food for the full development of the maggots. Faeces-staining, presence of exudate due to a prior lesion, and products of wool hydrolysis have been shown to be adequate to carry the larvae through the first stage. Wool hydrolysis on the living sheep is probably not an important factor, judging by an examination of wool samples, but we have isolated organisms which, when growing on a nutrient medium, are capable of disintegrating wool fibre.

5. The rôle of bacterial activity in strike is complex and appears to be substantially as follows:
(a) to produce substances which attract the flies and stimulate them to oviposit;
(b) to provide food for the initial growth of the maggots, either by rendering assimilable the inert proteins, or by causing a skin reaction with a serous exudation.

6. The immediate work for the future is a more exact determination of:
(a) the nature of the food of the larvae in the early stage of growth on the living sheep;
(b) the factors which influence its production; and
(c) the mechanism by which the larvae invade the skin.

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