STUDIES ON THE NUTRITION OF BLOW-FLY LARVAE

III. THE LIQUEFACTION OF MUSCLE

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INTRODUCTION.

Blow-fly larvae can only ingest liquid or semi-liquid food, since they have no teeth within the mouth. The present paper deals with the method by which these larvae obtain "liquefied" food from muscle. Guyenot (1907) suggested that blow-fly larvae feed on the liquefaction products of bacterial action. However, the investigations described here do not support this hypothesis. The contents of the fibres in raw meat are semi-liquid, and the feeding process appears to be largely mechanical. Predigestion of the structural elements of muscle may be necessary; if so, this can be accomplished by the larval excreta which have been found to digest proteins, including collagen, at an alkaline reaction (Hobson, 1931, 1931 *a*). The species of blow-fly larva used was *Lucilia sericata* Meig.

OBSERVATIONS AND EXPERIMENTS.

(1) Method of feeding on meat.

The atmospheric humidity largely determines the amount of decomposition and liquefaction in blown meat. Under moist conditions the surface rapidly becomes brown and liquefied, and eventually, if there are enough larvae, the meat is reduced to a dark brown liquid or froth. At low humidities the appearance of the meat is entirely different; the outside then dries to form a leathery case, under which a high concentration of ammonia develops and a skeleton of dried connective tissue remains where the larvae have been feeding. Although there is no evident liquefaction, the larvae grow rapidly and appear to be ingesting unchanged food.

Feeding habits of Lucilia *larvae.*

The emerging larvae feed for a short time on the lower surface of the meat, where the eggs are laid in large clusters; subsequently they bore upwards, choosing the comparatively fresh food within rather than the decomposing material on the outside. The larvae usually concentrate in large numbers at favourable spots and feed continuously. The process of feeding is accompanied by vigorous movements of the head and is not merely a passive act of sucking. Weismann (1864) has given an excellent description of the mouthparts and head armature of blow-fly larvae. The chief points of interest are the strong hooks which are articulated and lie outside the mouth, the powerful structure of the head armature and the size of the attached muscles; there are no teeth inside the mouth. Weismann regarded the head armature solely as an organ of locomotion, for crawling and boring through the food; however, its main function may be to macerate the food, for which it is clearly well adapted.

It was not possible to observe the feeding process in minute detail as the larvae move away from bright light, but the following points were noted. The larvae attack the fibres, even when free juice is present, and employ a typical scraping action which also occurs when they are crawling over glass. The head is first retracted, then moves forwards and upwards, and finally strikes down on to the surface. When feeding under normal conditions, the larvae pack side by side with their bodies still and their heads in rapid movement; the mass effect of this action must be considerable and may reduce the meat to a pulp. The larvae do not salivate freely on to the food, but they occasionally eject a small drop of saliva when crawling over glass.

Nature of the crop contents.

In order to determine the precise nature of the ingested food, the crop contents were examined. It may be noted that no digestion occurs in the crop, the salivary glands being devoid of proteolytic enzymes (Hobson, 1931). For these experiments larvae were reared until nearly full grown without changing the meat, unminced raw beef being used. Since haemoglobin, the pigment of red muscle, blackens when digested by proteolytic enzymes, the colour of the food in the crop serves as a rough index of the amount of predigestion which has occurred. When larvae are reared under dry conditions, their crops appear bright red, while under moist conditions, which favour bacterial action, the colour is usually brownish red and is only black when the meat has been consumed almost completely. In the following examination of the crop contents, larvae reared under moderately dry conditions were used.

The food present in the crop is a cloudy, viscous jelly having about the consistency of treacle. When mixed with water, it set instantaneously to a flocculent solid which could readily be dispersed to form a suspension; heating or addition of dilute acid coagulated the suspended precipitate. As the acid coagulum was insoluble in excess of the reagent, the crop contents do not consist of meta-protein which is insoluble only at its isoelectric point. To test for the presence of digestion products such as proteose or peptone, the crop contents of 10 larvae were dispersed in 1 c.c. of water and the mixture made acid with acetic acid, boiled and filtered. The filtrate, when tested with tannic acid, gave only a faint turbidity or a slight precipitate which was very small compared with the heat coagulum. The food in the crop was not appreciably soluble in 5 per cent. sodium chloride or 10 per cent. ammonium chloride solution. Saxhl (1907) found 65 per cent. of the protein of rigor muscle insoluble in saline. The crop contents therefore resemble muscle protein chemically. This evidence suggests that proteolytic action need not precede the ingestion of liquefied food from meat by larvae.

The food in the crop is not a protein sol, as it was found to flocculate in water, saline or dilute alkali; hence, it is probably a gel or colloidal suspension. The saliva may act as a protective colloid, as human saliva readily mixed with the crop contents. There is no appearance of definite structure either in fresh material or in fixed and stained preparations.

(2) Conditions necessary for the ingestion of liquefied food.

Since the food ingested by larvae differs from meat chiefly in physical properties, there appear to be two possible explanations of its formation, which are not mutually exclusive: (*a*) by mechanical action on the part of the larvae, (*b*) by physico-chemical changes not involving solution of the protein.

Effect of the alkaline reaction.

The mechanical hypothesis is supported by the chemical properties of the crop contents, the strength of the head armature and the vigorous nature of the feeding process. However, evidence that physico-chemical changes play an essential part has been derived from experiments with sterile muscle which will be described in a later paper (Hobson, 1932 *a*). In the absence of bacteria, the emerging larvae suck fluid for the first 2 days, the muscle remaining almost unchanged; later the reaction becomes alkaline, the surface of the muscle appears softened, and the typical semi-liquid jelly occurs in the crop. When reared on infected muscle, the larvae ingest semi-liquid food throughout. Preliminary changes in the muscle are therefore necessary and these may result either from larval or bacterial action.

With regard to the chemistry and structure of meat, Moran and Smith (1929) have recently investigated the changes occurring in muscle after death, and the following description is derived from their account. A muscle consists of a network of connective tissue binding together the muscle fibres which form its main bulk; the connective tissue, which is composed largely of collagen, occurs as fine fibres which ramify in all directions to form a continuous tough membrane. A delicate elastic sheath (the sarcolemma) encloses each muscle fibre, whose contents (the muscle substance) are semi-liquid in consistency and appear to possess a definite arrangement. Moran and Smith conclude that normally two processes occur during rigor mortis: (*a*) gelation of the muscle-plasma, (*b*) the production of acid which coagulates the protein gel and liberates an acid serum. Resolution of rigor is due to the slow breakdown of the coagulum by autolytic enzymes and the high concentration of lactic acid. The formation of acid is not essential for the occurrence of rigor; in animals depleted of glycogen, the precursor of the lactic acid in muscle, the onset of rigor is almost immediate after death and the duration is short. Normally rigor reaches its maximum in about 24 hours and resolution takes several days.

Moran and Smith describe the muscle substance in living tissue as "a very viscid material, which behaves in many respects like thick treacle." This description also applies to the crop contents of larvae feeding on meat under dry conditions. Since the acidity of dead muscle appears to prolong rigor and causes the separation of serum, the alkaline reaction of blown meat is likely to be a significant factor in the liquefaction of the muscle substance, provided the changes resulting from the acid reaction are not entirely irreversible. Experiments were therefore carried out to determine the influence of ammonia on the feeding of larvae on muscle; these showed that the reaction determines the type of food ingested.

Half-grown larvae were starved until their crops were empty, placed on the food and examined at intervals. As post-rigor muscle, fresh lean beef was used; rigor muscle was dissected from a frog killed the previous day. The muscle was made alkaline by exposing it to ammonia vapour until the *p*H was approximately 8·5, the reaction of blown meat. This treatment produced a striking change in the physical properties of the muscle. It became hard, felt quite dry to the touch and left no stain when pressed on filter paper; the untreated muscle appeared moist and drops of liquid could readily be expressed from it. Tests were also made with meat, which had been freed as far as possible from juice by pressing on filter-paper and drying for a few minutes in a warm incubator. The results are given in Table I.

Table I. *Nature of the food ingested by* Lucilia *larvae from muscle under different conditions.*

Medium	Reaction	Observations
Fresh meat (beef)	Acid	Larvae sucked fluid serum for several hours
One-day-old muscle (frog)	Acid	Larvae sucked fluid serum for several hours
Ammoniated meat	Alkaline	Crops were rapidly filled with a thin jelly which did not dissolve in water
Ammoniated frog muscle	Alkaline	Larvae sucked liquid for 10–15 min.; then a white coagulum began to appear in the crop. After 40 min. feeding the crop contents were an insoluble jelly. In larvae removed from the muscle after 10 min. feeding, the crop contents remained liquid and did not coagulate
Meat pressed to remove juice	Acid	Larvae sucked juice at first; after 20 min. they began to ingest muscle substance which appeared in the crop as a white coagulum

These observations show that larvae can ingest the muscle substance only when the serum has been removed. The effect of making the muscle alkaline is presumably due to absorption of the free liquid by the contents of the fibres; also, the hardness of ammoniated muscle may up to a point assist the mechanical action of the larvae by providing resistance. Although this evidence is not quantitative and refers to cut surfaces, it suggests that the chief factors concerned in the ingestion of the muscle substance are the mechanical action of the larvae and the presence of ammonia. Digestive action probably assists in the feeding process, as the larval excreta have been shown to be strongly proteolytic (Hobson, 1931).

Extra-intestinal digestion.

That the enzyme in the excreta comes into contact with the food was shown by removing larvae and rinsing the anterior part of the body; the washings were found to liquefy gelatine rapidly. However, autolysis experiments indicated that the concentration of enzyme in the ingested food is not appreciable; for, when larvae were killed by drowning, the crop contents remained almost unchanged for several days. The main function of the excreted enzyme may be to digest the structural elements of muscle, the intra-muscular connective tissue and the sarcolemma of the fibres. This tissue is very small in amount compared with the muscle substance and would be the first to come into contact with the enzyme. It has already been shown that the excreta of aseptic larvae contain protease and collagenase, also free ammonia (Hobson, 1931 *a*, 1932).

Digestion of the outer cover of the fibre probably supplements mechanical tearing, for which the hooks of the larvae are well adapted. Once the muscle substance is exposed, maceration and ingestion may proceed rapidly; this would explain the low concentration of enzyme in the crop contents. However, if softening of the muscle substance is at all necessary and has not already resulted from bacterial action, the excreted protease is available for this purpose. Bacteria probably play an unessential part in the liquefaction of blown meat, except in the early stages when the larvae are very small and the muscle is still acid. Formation of ammonia is probably a more important function than proteolytic action; for, in regard to enzyme production, the larvae offset their small size by concentrating in large numbers; on the other hand, the ammonia in their excreta will tend to diffuse away, and similarly that produced by bacterial action will spread through the meat. The alkaline reaction of blown meat affects the feeding of the larvae in two ways, by activating the enzymes in the excreta and by changing the colloidal properties of the proteins.

DISCUSSION.

(1) Choice of food in blow-fly larvae.

Although *Lucilia* larvae grow normally in a decomposing medium, they appear to prefer relatively fresh food and are often injured by excessive putrefaction. Bogdanow (1908) found several organisms toxic in pure culture to *Calliphora* larvae on sterilised meat. These observations are of some interest in view of the work of Holdaway (1930) on the fauna of exposed meat and carrion. A definite ecological succession was found to occur among the different species of blow-fly larvae and other insects, the nature of the fauna being partly determined by the type and rapidity of putrefaction. *Lucilia sericata* acts as coloniser, the females laying eggs on fresh food shortly after exposure. Larvae of Sarcophagids and *Chrysomyia* appear later when liquefaction has begun; *Calliphora* species are intermediary.

With regard to myiasis, it may be noted that *Lucilia* and *Calliphora* larvae feed only on dead tissue according to Baer (1931); his observations refer to maggots used therapeutically in the treatment of osteomyelitis, large numbers being placed in open wounds. Since my experiments have shown that larvae feed readily on sterile muscle, products of bacterial action are not essential as attractants. There appears to be no published[1] evidence as to the ability of these larvae 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.

(2) THE LIQUEFACTION OF MUSCLE.

Liquefaction, whether by micro-organisms or blow-fly larvae, differs from normal proteolytic digestion in that the amounts of enzyme and free liquid are relatively small compared with the mass of substrate. The initial product resembles a jelly and may result from solution of water in protein together with a breakdown of the structural elements. Examination of the crop contents of *Lucilia* larvae has shown that extensive proteolytic action is not essential for the ingestion of liquefied food from meat. In this respect the process of liquefaction bears some resemblance to the resolution of rigor mortis; for, in both, softening of the muscle substance can occur without the formation of marked amounts of soluble products, and enzymic digestion is not the only factor involved. In the liquefaction of meat by larvae, the alkaline reaction produced alters the colloidal properties of the protein to a considerable extent.

Feeding experiments showed that previous treatment of muscle with ammonia prevents larvae from sucking the serum; this is presumably due to absorption of the free liquid by the muscle substance. It is well known that most proteins imbibe water and swell if the reaction is moved in either direction away from the isoelectric point. Although the acid reaction of dead muscle tends to denature the coagulated protein, the process is not complete, as von Furth and Lenk (1911) found that post-rigor muscle swells in acid solution, though not to the same extent as pre-rigor muscle. Lloyd (1926) showed that muscle swells in acid and alkaline solutions and that the imbibitional power of tissues is limited by their structure, the resultant turgor opposing the increased osmotic pressure. However, this will not prevent the absorption of the small amount of free liquid in dead muscle when exposed to ammonia gas. Lloyd found minimum swelling of muscle between pH 5·0 and

[1] In a private communication Dr G. Salt informed me that he carried out some unpublished experiments on this point at the Farnham House Laboratory. He found that *Lucilia* larvae can penetrate the skin of recently killed, small animals.

7·0; the reaction of fresh meat lies between these limits and, when blown, rapidly becomes more alkaline than *p*H 8·0.

Exposure of muscle to ammonia gas produces an extreme rigor which may be partly due to turgidity; the alkaline reaction may also produce intrinsic changes in the muscle substance. Edsall (1930) studied the effect of alkali on isoelectric muscle globulin; when the gel was titrated with soda, its rigidity continued to increase until the *p*H reached 8·5; the rigor noted in dead muscle at this reaction may be a similar effect.

SUMMARY.

1. Investigation of the feeding of *Lucilia* larvae on meat suggested that the chief factors involved are mechanical maceration and the alkaline reaction which results in the first place from bacterial action.

2. Larvae suck the fluid serum from acid muscle; they ingest semi-liquid food only when the reaction is alkaline or the free liquid has been removed.

3. Predigestion of the muscle substance is apparently not essential, as the crop contents often consist of insoluble protein.

4. The proteolytic enzymes in the larval excreta, which include a collagenase, probably serve to digest the structural parts of muscle tissue.

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