THE RATE OF PRODUCTION OF THE PERITROPHIC MEMBRANE IN SOME INSECTS

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Summary

Feeding zygopterous (damsel-fly) nymphs produced peritrophic membranes at a rate of about three per day. Each membrane is secreted by the entire midgut epithelium and varies in length according to the degree of distension of the midgut by food. Starving nymphs evacuated empty membranes at a slower rate (about 1 per day) until death.

Larvae of the drone-fly *Eristalis tenax,* when transferred to fresh water, evacuated their continuous, tubular peritrophic membrane for some hours at 30°C at a rate of about 5 mm/hr. After 42 hr without access to food the rate dropped to about 2 mm/hr. The corresponding production rates for larvae in dilute, filtered liquid manure were 6 and 3 mm/hr respectively. Food passed through the alimentary canal of actively feeding larvae at a rate varying from 50 to 75 mm/hr and therefore travelled at a very much greater rate than that at which the peritrophic membrane was produced.

In blowfly larvae, removed from their food and kept at 30°C, the continuous peritrophic membrane was produced at a rate varying between 5 and 10 mm/hr for the first 24 hr. Food passed down the alimentary canal at about 75 mm/hr.

Well-fed adult earwigs discharged their peritrophic membrane at an average rate of about 1.6 mm/hr at 30°C. Food passed along the alimentary canal at this rate or slightly faster. At about 18°C fed individuals produced their membrane at about 0.25 mm/hr. During starvation at 30°C the rate of membrane production fell to 0.6 mm/hr.

I. INTRODUCTION

Although much has been written on the occurrence of the peritrophic membrane (PM) in insects and on its mode of production, there is very little information available on the rate at which it is produced or on how this rate is influenced by the intake of food.

The PM is produced in some insects by a ring of cells at the anterior end of the midgut (type I) and in others by the delamination of one or more layers from the surface of the midgut epithelium (type II). Whatever the method of production, the PM appears to be present continuously in most insects which produce it, although in some, e.g. adult mosquitoes (Waterhouse 1953), it has only been detected after feeding. Since fragments of membrane are present in the faeces of most insects it follows that the PM is either produced continuously or at intervals and in either case is passed on with the food.

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Where the membrane is produced at the anterior end of the midgut it is generally thought to be passed on at about the same rate as the food. This is suggested by observations, such as those on the tsetse fly, *Glossina* (Wigglesworth 1929), in which the PM in the freshly emerged adult is ragged and discontinuous. Immediately the first meal is ingested the PM is perfectly formed and extends to the limits of the ingested blood. Further, the data on the development and distribution of trypanosomes within the fly can be explained only if the PM is not penetrated by trypanosomes and if it extends continuously as far as the middle of the hindgut before infected blood has reached this level (Hoare 1931; Yorke, Murgatroyd, and Hawking 1933).

The only numerical data on the rate of production of the type I PM is that of Aubertot (1932*a*) who observed that a single *Eristalis tenax* (L.) larva, placed in fresh water for 17½ hr, produced a PM at the rate of 6.1 mm/hr.

There is very little more known on the rate of formation of the delaminated type of PM, where the information required is the frequency with which the layers comprising the PM are formed. Rengel (1903) reported that at least five to six membranes were delaminated by wasp larvae (*Vespa*) and an even larger number by honey-bee larvae. The rate could be measured because the food residues are retained in the larval midgut until pupation. As food continues to be ingested the innermost membranes burst, whereas the outer, larger, more recently formed membranes remain intact. It is possible, therefore, to make an estimate of the number of layers of membrane at various stages of larval development. Aubertot (1932*b*) recorded that a single starving *Aeschna* (dragonfly) larva produced PM's at the rate of two per day for 4 days. In this instance the number of membranes produced could be recorded since, at intervals, the midgut contents (together with their enveloping PM) are passed rapidly down the hindgut and excreted.

The principal difficulty in measuring the rate of production of the PM, except in such specialized forms as larval Hymenoptera, is to recover it in sufficiently undamaged form from the faeces. Although the relatively tough type I membrane is produced by many insects it is frequently broken into small pieces in the hindgut before discharge. Thus, in adult blowflies the rectal papillae carry backwardly directed spines which break up the membrane so effectively that only fragments can be recovered from the faeces. In insects which produce the delaminated type of PM the faeces are frequently solid or semi-solid. When the food residues, enclosed in the PM, are passed into the hindgut (e.g. *Locusta, Periplaneta*) the PM is fragmented during the formation of the faecal pellets, so that it is difficult to relate the length of the faecal pellet to the length of PM originally enclosing the corresponding mass of food in the midgut. Furthermore this gives no direct information on the frequency of delamination from the surface of the midgut epithelium.

The formation of solid or semi-solid faecal pellets is often related to the insects' need to conserve water. It is noteworthy, for example, that the PM is discharged in a relatively undamaged condition by many aquatic or semi-aquatic insects and it is these insects which appear to provide the best material for measuring its rate of production. Another advantage in using aquatic larvae

is that the task of recovering for examination the transparent, almost invisible PM is greatly simplified if it remains suspended in water. For these reasons it was decided to use two aquatic insects (nymphs of *Ischnura heterosticta* Burm. (Odonata), larvae of *Eristalis tenax* (L.) (Diptera : Syrphidae)) and three insects which feed on moist food (larvae of *Lucilia cuprina* (Wied.), *Calliphora augur* (F.), and *C. fallax* Hardy: Diptera : Calliphoridae). The earwig *Labidura truncata* Kirby was also used. Dermaptera produce the inner portion of their PM from cells at the level of the oesophageal invagination and the outer layers by delamination from the general midgut epithelium. This then represents a type intermediate between the Odonata and Diptera examined. Information was particularly desired on the rate of production (or delamination) in feeding and starving insects and on the relative rates of passage of food and PM along the alimentary canal.

II. Methods and Material

(*a*) *Odonata*

Nymphs of several species of Odonata (mainly belonging to the sub-order Zygoptera (damsel-flies)) were collected from a stream and kept individually in small glass vessels in fresh water to a depth of 5 or 6 mm. The most abundant species was *Ischnura heterosticta* Burm. (Coenagriidae), although *Austrolestes cingulatus* Burm. (Lestidae) and *Austroagrion cyane* Selys. (Coenagriidae) were present in some batches of nymphs. All species of Zygoptera appeared to behave similarly. The nymphs were either starved or offered an abundant supply of mosquito larvae (*Aedes aegypti* L. or *A. notoscriptus* Skuse). A multilayered PM varying in thickness from about 0.5 to 2.0 μ is formed in the midgut and is retained therein for some hours before being passed on *in toto* to the hindgut. Up to eight layers have been distinguished in membranes from feeding nymphs, although three or four is a more usual number. However, it is not possible to estimate the number of layers at all accurately. In fact it is generally only when some of the component layers become fortuitously separated that it is possible to make out the individual layers at all clearly. In Odonata the rectum has an important respiratory function and the membrane is only permitted to remain in the hindgut for a relatively short time before being evacuated and hence is discharged in an undamaged state.

Both number of multilayered membranes produced and their length were recorded at regular intervals and, in addition, a record kept of the consumption of mosquito larvae in order to indicate whether the nymphs were in an actively feeding condition.

(*b*) *Diptera*

Larvae of *Eristalis tenax* (L.) (Syrphidae) were obtained from liquid horse manure both from a laboratory culture and from the field. Larvae ranging in length from 10 to 15 mm (to the base of the respiratory siphon) were used in most experiments, although slightly smaller or larger larvae were employed occasionally. These lengths are only approximate, since it is very difficult to make measurements with any accuracy when the larvae are actively extending

and contracting their bodies. The larvae were washed in fresh water and placed in squat 10-ml beakers containing water or coloured liquid manure to a depth of about 1 cm. Very few larvae escaped so long as there was sufficient fluid to cause them to swim and provided that the diameter of the container was greater than the length of the body.

The tubular continuous PM projected from the anus as it was excreted, pieces of variable length (from a millimetre or so to up to 60 mm) being broken off intermittently and fortuitously by larval movements. For measurements of rate of production, pieces of extruded membrane which had not been detached by the larvae were broken off as close as possible to the anus. Care had to be exercised to prevent several additional millimetres of membrane being pulled out of the anus in this process. The length of the pieces of membrane was measured under water, using a pair of adjustable dividers and a magnification of 6.6×. There were naturally errors in estimating the length of the fragments of PM, since it was difficult to get them to lie straight. Where several lengths from a single larva were measured at the same time it was found convenient to record each by pricking it on a ruled index card and to measure the total length produced. This procedure reduced the error in estimating fractions of a millimetre. The total lengths of membrane were recorded to the nearest 0.5 mm.

Almost fully grown third-instar blowfly larvae (*Lucilia cuprina* (Wied.), *Calliphora augur* (F.), and *C. fallax* Hardy) were removed from liver and, after washing, transferred to excavated blocks containing a few drops of water. The top of each block was covered with a "Perspex" lid in which several holes 1 mm in diameter had been bored. The larvae carried a film of water with them as they moved and this soon sealed the lid to the excavated block. The holes in the lid, however, enabled an adequate supply of oxygen to reach the larvae. The lengths of membrane produced were measured as for *E. tenax*.

(*c*) *Dermaptera*

Specimens of *Labidura truncata* Kirby were collected in the field and maintained individually in glass-covered excavated blocks. They lived well on "Farex*" moistened with water and this food resulted in a humidity approaching saturation being maintained in these containers, which prevented the faeces from drying out. The faecal pellets were transferred to water for examination. The pellets varied from a single short piece of membrane surrounding food residues to a group of half a dozen or more such pieces, some lying longitudinally and others transversely to the hindgut axis. Each of these pieces is detached from the remainder of the membrane in the hindgut just anterior to the rectum. When the pieces of membrane had been crumpled during passage through the hindgut, or if they were lying transversely in the faecal pellet they were carefully teased out and laid end to end before measuring. These measurements were undoubtedly subject to some error because it is not possible to reconstitute with a high degree of accuracy several dozen pieces, mostly

* A proprietary baby food: Glaxo Laboratories (Aust.) Pty. Ltd.

varying from 0.5 to 1.5 mm in length. However, it is believed that the errors involved did not materially affect the conclusions reached.

TABLE 1

RATE OF PM PRODUCTION IN FED ZYGOPTEROUS NYMPHS

	No. PM's Produced		*A. aegypti* Larvae Eaten
	0-24 Hr	24-48 Hr	0-48 Hr
1	4	3	7
2	4	4	13
3	4	2	4
4	3	4	12
5	3	4	6
6	2	3	7
7	5	2	10
8	3	4	6
9	3	3	9
10	3	2	4
Total	34	31	71

III. RESULTS

(*a*) *Zygoptera*

(i) *Rate of PM Production in Fed Nymphs.*—Ten nymphs varying in length from 10 to 17 mm (excluding the anal gills) were continuously supplied with *A. aegypti* larvae. The results (Table 1) show that, in these actively feeding nymphs, two to five multilayered membranes were produced per day with an

TABLE 2

INFLUENCE OF FEEDING AND STARVATION ON RATE OF PM FORMATION IN ZYGOPTEROUS NYMPHS

Series	No. Larvae	Nutrition	No. Days	PM's/Day
1	7	Fed	5	1·5
	6	Starved	4	1·2
	3	Fed	6	1·9
2	6	Starved	5	0·8
	3	Fed	7	2·7
	2	Starved	3	1·3

average of 3.3 per nymph per day. Other observations also indicate that the normal daily production is usually in the vicinity of three membranes per nymph per day, although rates as low as 1.5 per day have been recorded (Table 2).

Although the average figures indicate that a membrane is discharged every 8 hr the intervals between the expulsion of successive membranes is rather irregular. The shortest interval recorded for fed nymphs was 2 hr.

(ii) *Effect of Starvation on Rate of PM Production.*—Two series of 10 nymphs were set up in individual containers and subjected to alternating periods of feeding and starvation, each period lasting several days (Table 2). The number of nymphs finally considered was less than the original 20. This is because figures for nymphs which moulted, died, or emerged during any one period were excluded from the records.

TABLE 3

RELATIVE LENGTHS OF PM'S AND MIDGUT OF ZYGOPTEROUS NYMPHS

Length of Midgut (mm)	Length of PM's (mm)
4·5	6·5
2·5	3·0
3·0	3·0, 3·0, 3·0 (empty), 5·5, 5·5 (full)
3·5	4·0, 4·0, 4·5, 4·0, 3·0, 2·5
2·5	2·5, 2·0

In both series the frequency of PM discharge was lower in starved than in fed nymphs. Once the starving phase had been initiated and the food residues in the digestive tract eliminated, the nymphs produced transparent empty membranes. There was no consistent downward trend in the rate of production over any one period of observation.

TABLE 4

LENGTH OF PM'S DISCHARGED BY INDIVIDUAL ZYGOPTEROUS NYMPHS

	Length of PM (to nearest 0·5 mm)	Range	No. PM's
1	4·5, 3, 6, 6·5, 4·5, 7, 9, 4·5	3-9	8
2	5·5, 7·5, 5, 8, 5, 8, 7, 7, 4, 4, 4, 4, 4·5, 7, 3·5	3·5-8	15
3	4, 7, 7, 4, 3·5, 6	3·5-7	6
4	5·5, 6·5, 6·5, 6·5, 8, 6·5, 6, 7, 7, 6·5, 5·4, 3·5	3·5-8	12
5	3, 5, 5, 4, 4·5, 4·5, 5, 4	3-5	8
6	7, 7, 5, 5·5, 7·5, 5, 5, 5, 8, 5·5, 3, 6·0	3-8	12
7	4·5, 5, 9, 9, 5·5, 6, 6·5, 6, 6, 4·5, 5, 2·5	2·5-9	12
8	4·5, 4, 4, 3·5, 3·5, 3, 2·5, 2	2-4·5	8
9	3, 4, 4·5, 4·5, 3·5, 4·5, 3	3-4·5	7
10	2, 3, 3, 2·5, 2·5	2-3	5

(iii) *Relation of Length of PM to Length of Midgut.*—Five nymphs were dissected after they had produced one or more membranes and the lengths of the PM's and midguts measured (Table 3). Records were also kept of the lengths of successive PM's produced by 10 nymphs (Table 4).

It is clear from these figures that there is a very considerable variation in the lengths of successive PM's produced by the same larva. Furthermore, the lengths may be considerably greater than, equal to, or slightly less than the length of the midgut as measured on dissection. This variation is undoubtedly due to the great capacity of the midgut for distension. The lengths of the PM's produced by starving nymphs were found to be relatively constant and to approximate closely to the length of the midgut. However, since food is taken intermittently (even if it is continuously available) and since the size of the meal varies enormously (from a single small mosquito larva to two or three large larvae) the distension of the midgut varies accordingly and membranes of variable length are produced as a result.

(iv) *Rate of Passage of Food.*—Some finely divided food material was observed to have reached the posterior end of the midgut of a previously starved nymph 4 or 5 min after a mosquito larva had been caught. However, the main bulk of a meal is frequently retained in the crop for some hours before being passed on to the midgut. The average time of passage of food through the alimentary canal is in the vicinity of 8 hr. As mentioned earlier, the shortest time recorded between the evacuation of successive membranes was 2 hr, so that food on this occasion passed through midgut and hindgut in this period.

(*b*) *Anisoptera*

A few observations on unidentified dragonfly nymphs indicated that these behaved in a fashion similar to damsel-fly nymphs. Membranes, which varied rather widely in length, were produced at a rate of about 1.8 per day. The most rapid rate of production observed was two membranes within 5 hr.

(*c*) Eristalis *Larvae*

E. tenax larvae do not possess a storage crop to maintain for some time a continuous supply of food to the midgut when larvae are removed from their food medium. Under natural conditions they ingest food continuously. When placed in fresh water therefore, as in some experiments, the rate of passage of food and the rate of production of the PM might be expected to be influenced by this somewhat unnatural environment.

(i) *Rate of Production of the PM*

(1) *In Fresh Water.*—The rate of production of the PM was investigated at 2-hr intervals for 6 hr after larvae had been transferred to fresh water.

As can be seen in Table 5, there was a great individual variation, not only in the total length of PM produced (from 11.0 to 47.0 mm) by the various larvae over the 6-hr period, but also in the lengths produced during any 2-hr period. Undoubtedly one of the factors involved in the very variable rate of production by the same larva is that the PM is coiled upon itself in various regions of the gut. Lengths of membrane longer than usual may then be discharged at irregular intervals either naturally or when the larvae are stimulated by handling. During the first 2-hr period the larvae produced lengths of PM at the rate of

5.3 mm/hr. The rate dropped during the second 2-hr period to 3.7 mm/hr and rose during the final 2-hr period to 5.4 mm/hr, with an overall production rate of 4.8 mm/hr. The greatest individual production rate was observed during the 4-6-hr period when a single larva produced a 29-mm length of membrane.

In spite of the variability encountered it is clear that the PM is produced continuously and at a fairly constant rate, at least for the first 6 hr after removal from food.

TABLE 5

RATE OF PRODUCTION OF PM BY *E. TENAX* LARVAE SUSPENDED IN FRESH WATER AT 30°C FOR 6 HR AFTER REMOVAL FROM LIQUID MANURE

	Larval Length (mm)	Length of PM Produced (mm)			
		0-2 Hr	2-4 Hr	4-6 Hr	Total for 6 Hr
1	10	7·5	0	6	13·5
2	12	10	11·5	13·5	35·0
3	13	3·5	5	2·5	11·0
4	13	5·5	4	21	30·5
5	13	6	2	3	11·0
6	14	19	5	15	39·0
7	14	8	9	3	20·0
8	15	7	2·5	29·0	38·5
9	16	24·5	5	9·5	39·0
10	16	14·5	12	13	39·5
11	16	9·5	7	3·5	20·0
12	16	2·5	13	5	20·5
13	17	0	11·5	5	16·5
14	17	17·5	8	13	38·5
15	17	14	19·5	13·5	47·0
16	18	7·5	5	17·5	30·0
17	19	24	6	9·5	39·5
Totals		180·5	126·0	182·5	489·0
Rate per larva per hour		5·3	3·7	5·4	4·8

(2) *In Filtered Liquid Manure.*—It was necessary to determine whether the rate of production was appreciably lowered in the above experiment because no fresh food was being ingested by the larvae during the period of test.

Larvae were therefore set up in diluted, filtered liquid manure to determine whether the production rate was greater under these conditions than in fresh water (Table 6). The suspending medium had to be free from solids to enable all PM fragments to be recovered.

The results clearly indicate that the average production rate is higher in larvae living in liquid manure than in water. The drop in the rate of PM pro-

duction between 2 and 4 hr and its subsequent rise between 4 and 6 hr (also recorded in Table 5) are at present unexplained. The production rate fell gradually after the first 6-hr period, but it is clear that the larvae continued to secrete a membrane over the entire experimental period. The greatest individual production rate recorded in these experiments was 36 mm between 4 and 6 hr for a larva in fresh water.

It is unfortunately not possible to determine whether the PM production rate is still higher when larvae are feeding in their more normal semi-solid medium, because of the difficulty of recovering all lengths of peritrophic membrane. However, from the fact that the rate of production is not very different for the first 10 or 15 min after removal from their natural food than for the next couple of hours, it seems unlikely that the average production rate would exceed 10 mm/hr. Arguing similarly, the maximum production rate might possibly be 30 mm/hr instead of the maximum of 18 mm/hr observed.

TABLE 6

PRODUCTION OF PM BY 10 *ERISTALIS* LARVAE SET UP IN FRESH WATER AND IN DILUTE FILTERED LIQUID MANURE

	Fresh Water					Dilute Filtered Liquid Manure				
	0-2 Hr	2-4 Hr	4-6 Hr	6-24 Hr	24*-48 Hr	0-2 Hr	2-4 Hr	4-6 Hr	6-25 Hr	25-49 Hr
Total length produced (mm)	91	72	100	492	429	122	56	122	698	611
Rate per larva per hr (mm)	4·6	3·6	5·0	2·8	2·2	6·1	2·8	6·1	3·7	2·8

* Two larvae died during this period and were excluded from the calculations.

(ii) *Rate of Passage of Food*

The most rapid rate of food passage from mouth to anus observed at 30°C was 80 min for several larvae transferred to liquid manure containing finely powdered animal charcoal. One of these larvae was approximately 11.5 mm long and had a total gut length of 66 mm. Two other larvae were 14 mm long and each had a gut length of approximately 100 mm. The food, therefore, passed through the alimentary canal in these larvae at a rate between 50 and 75 mm/hr.

In a number of other larvae tested either with charcoal or finely divided Prussian blue as markers, food had passed through to the anus in 85-120 min. On the other hand in some larvae the coloured food had only travelled as far as the middle or end of the midgut after 2 hr and in others it had not quite reached the end of the hindgut after 24 hr. There are evidently variations in

the rate of passage of food which may be due to individual variation between larvae or related to differing physiological states.

When active larvae were suspended in fresh water the majority had discharged all food residues from the gut in 6 hr or less.

(iii) *Relative Rates of Food Passage and Membrane Production*

When the deduced maximum rate of PM production of about 30 mm/hr is compared with an observed rate of passage of food of 50-75 mm/hr it is apparent that the food must often pass down the alimentary canal at an appreciably greater speed than the PM. This conclusion is supported by many observations of larvae which have caused considerable fouling of the fresh water in which they have been placed without extruding more than a few millimetres of membrane during the same period. Furthermore, when larvae had been transferred from liquid manure + carbon to liquid manure + congo red, particles of carbon could be detected within the PM more than 1 cm behind the advancing front of the congo red-stained food, suggesting that they had been left behind the main mass of carbon-marked food. It was observed in these experiments that the PM was permeable to congo red, which is what might have been expected from similar results with adult *Calliphora* (von Dehn 1933).

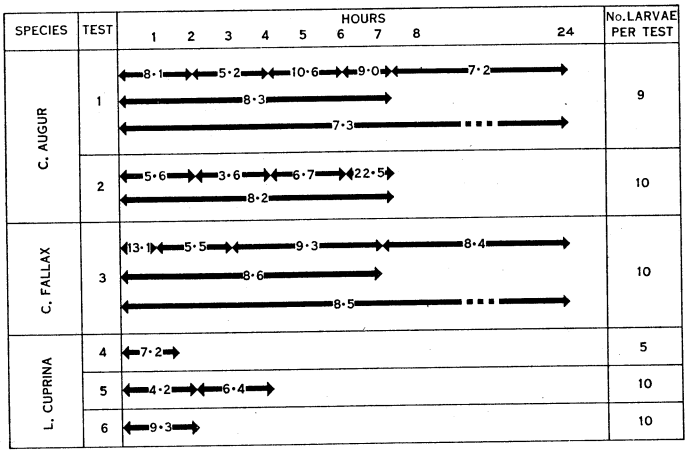

Fig. 1.—Chart showing the average rate of production of PM (mm/hr) by calliphorid larvae at 30°C. The figure within a segment of horizontal line is the average rate for the period of hours (after removal from food) covered by that segment.

(*d*) *Blowfly Larvae*

(i) *Rate of Production of the PM.*—Figure 1 summarizes the results obtained for rate of PM production with three species of blowfly larvae. Although there was considerable individual variation in production rates, particularly over shorter periods of time, the average rate for all three species generally fell within the range 5-10 mm/hr. Rates for the first 7 (or 7¼) hr for the two *Calliphora* species varied from 8.2 to 8.6 mm/hr. There is no reason to believe

that this rate is appreciably less than in feeding larvae. The maximum rate observed was 67 mm in 1¼ hr (from 6 to 7¼ hr) for a *C. augur* larva. This was almost certainly due to the discharge of stored-up PM produced over a much longer period, since this larva had discharged membrane at only 4 mm/hr for the previous 6 hr. It is clearly necessary to measure production rate over a period long enough to average out such individual variations in discharge rate, but not long enough to extend much into the period when the production rate starts to diminish. The temporary reduction in rate of discharge between 2 and 4 hr, already noted for *Eristalis,* also occurred in these experiments.

(ii) *Rate of Passage of Food.*—Almost fully fed third-instar *L. cuprina* larvae were transferred to finely ground liver coloured with solid Prussian blue and maintained at 30°C. Measurements for these larvae were: weight 34 mg, total length 10 mm, foregut 3 mm, midgut 75 mm, hindgut 35 mm. In those which commenced to feed immediately after transfer, coloured food had travelled in 10-15 min some 40 mm to reach the middle region of the midgut. This region has, for a short distance, a greater diameter than the PM, which consequently coils up upon itself several times. If recently ingested food already occupies the middle region of the midgut coloured food is temporarily held in the anterior midgut. Coloured food usually passes no further than the mid midgut until some 50-60 min after transfer, when it moves down through the posterior midgut and hindgut to reach the anus about 90 min after ingestion. The larvae all had full crops when transferred and coloured food usually moved straight past the crop into the midgut. Occasionally, however, a small amount of this coloured food found its way into the upper third of the crop.

It is clear from these figures that the rate of passage of food along the digestive tract is many times greater than that at which the PM is produced, a result already noted for *Eristalis* larvae.

(*e*) *Dermaptera*

(i) *Rate of Production of the PM.*—Individual *Labidura truncata* varying in length from 13 to 21 mm (tip of head to base of forceps) were maintained at 30°C and supplied daily with fresh food. No important differences were observed in rate of PM production between males, females, and large nymphs.

In a typical experiment 2804 mm of membrane were produced in 1748 'earwig hours,' an average rate of 1.6 mm/hr. As an indication of the variation in production rate, the average rate for the first 958 hr of this period was 1.7 mm/hr and, for the second period of 790 hr, 1.4 mm/hr. Figures for the second period are shown in Table 7. The fastest individual rate over a 24-hr period was 3.4 mm/hr (for a nymph 13 mm long) and the slowest was 0.4 mm/hr (for a female 19 mm long). Values approaching these were, however, not common.

At room temperature (15-18°C) activity was restricted and feeding intermittent. Over a 13-day period a PM was produced at a rate of 0.25 mm/hr.

(ii) *Effect of Starvation on Rate of PM Production.*—Individuals were supplied with drinking water only (in small glass tubes) and maintained at 30°C.

For 48 hr before starvation commenced they produced PM's at a rate of 2.2 mm/hr. In the first 48 hr of starvation the rate fell to 0.9 mm/hr and levelled out at 0.58 mm/hr for the following 10 days. No deaths occurred during this period and the membranes produced were devoid of contents.

(iii) *Rate of Passage of Food.*—A starved *Labidura* commences to feed immediately food is presented. Food passes almost immediately to the posterior end of the crop and, at 30°C, within 30 min it has generally reached the end of the midgut. By 3 hr it may reach the rectum and faecal pellets may be produced from 4 hr onwards.

TABLE 7

PM PRODUCTION (MM) AT 30°C IN CONTINUOUSLY FED *LABIDURA*

Period (hr)	Length (mm) of Membrane Produced				
	1	2	3	4	5
24	60	30	34	34	61
24	22	30	39	14	23
7	13	8	10	2	12
24	33	58	30	24	59
24	32	39	34	56	31
24	16	17	15	29	11
24	19	31	32	81	14
7	12	9	11	14	14
158	207	222	205	254	225

The length of the alimentary canal varies somewhat according to the degree of distension by food. For individuals measuring 15-20 mm from the tip of the head to the base of the forceps the engorged foregut measures 15-19 mm in length, the midgut 4-6 mm, and the hindgut 7-9 mm. Assuming immediate passage of food into the midgut in starved individuals, passage of food along the 4-6 mm midgut length took half an hour (equivalent to a rate of 8-12 mm/hr). The most rapid recorded rate of PM production (for 24 hr) was 3.4 mm/hr, which suggests that the food in previously starved animals may be passed on more rapidly than the PM.

Rate of food passage in well-fed *Labidura* is appreciably slower than in previously starved individuals. Coloured faeces were not infrequently passed 7-8 hr after stained food was offered, a minimum of 9 mm of PM being discharged in this period. From these figures it appears that food normally passes through the alimentary canal at about the same rate as (or slightly faster than) that at which the PM is produced.

IV. DISCUSSION

Figures are now available to support the conclusion that the type I PM is produced continuously and at a rate which is higher in actively feeding than in starving insects.

By contrast, the midgut epithelium evidently produces the lamellae of the type II membrane intermittently, periods of secretion alternating with periods when the epithelial cells are elaborating further precursor materials. The intervals between periods of delamination are shorter, in the zygopterous nymphs examined, in fed than in starving insects. Unfortunately the individual lamellae are not only insufficiently separated, but are also too fine to be counted accurately, so that the frequency of each delamination has not been determined. It appears to be once every hour or two.

Evidence that the PM is produced by the entire midgut epithelium in zygopterous nymphs follows from the observation that the length of the PM corresponds with the length of the midgut. Only if the entire midgut produced the PM would its length vary in accordance with the varying degrees of midgut distension, the latter depending upon the size of the meal ingested.

A somewhat unexpected observation was that food passes down the digestive tract in the fly larvae examined much faster than the rate at which the PM is produced. The rate of passage of food must be governed to a certain extent by the length of the alimentary canal to enable at least a minimum time for digestion and absorption to occur. The rate is thus considerably less in *Drosophila* larvae where, however, food also passes from mouth to anus in less than 2 hr (Bowen 1951). The more rapid passage of food than membrane results in some food particles being left behind the main mass moving within the PM. With a rate of food passage of the order of 75 mm/hr it is possible that the PM does provide a useful measure of protection for the midgut epithelium which, in the larvae of many Diptera, is notable in that it is not replaced at moulting as in most insects. Furthermore, the continuously produced PM ensures that all food residues are passed down the digestive tract at a rate not less than that at which the PM is formed. In the absence of a PM it is probable that some residue would remain for relatively long periods near the surface of the midgut cells. That this may happen to materials which have passed out through the PM is shown by observations on *Drosophila* larvae (Bowen 1951). Much of the radio barium added to their diet may be held for 10-20 hr in the space between the PM and the anterior midgut epithelium, and no ^{140}Ba was found in this space in the lower regions of the midgut. The fact that the fluid filling this space (containing as it does the products of digestion) moves so slowly in relation to the rapidly moving contents of the PM must greatly increase the efficiency of absorption.

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