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Feeding and Crop Emptying in *Glossina brevipalpis* Newstead

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Introduction

In some laboratory bred *Glossina* the crop ruptures and the ingested blood escapes into the haemocoel. This phenomenon has also been reported in wild *G. brevipalpis* (BURTT, 1944). MEWS (1968) has described blood-clotting and crystals in the crops of fully engorged *G. morsitans* reared in the laboratory. Similar observations have also been noted in *G. brevipalpis* and *G. pallidipes* in our laboratory. Preliminary to an investigation of these abnormalities, information was needed on the structure and functions of the crop, proventriculus and its associated ducts in relation to the early movement of the blood meal.

The present study investigates the early course of the blood meal with particular reference to the structures involved and its relation to primary excretion.

Materials and Methods

Wild *G. brevipalpis* were caught in South Busoga, Uganda and used soon after they were brought to the laboratory. Laboratory reared flies were obtained from a colony maintained in an insectarium at 25°C, 80% R.H. and under a photoperiod of 12 h subdued natural light each day. Experimental flies were kept singly in 7.5 × 2.5 cm glass tubes, closed at one end with a cork and covered with mosquito netting at the other.

For observation on the general structure, flies at different stages of engorgement were dismembered and fixed in Carnoy's fluid or Bouin Duboscq. After fixation, the proventriculus with its associated ducts was either dissected out or left *in situ*. The fixed material was embedded in paraffin wax (m.p. 60°C) or double impregnated with celloidin followed by paraffin wax (CLAYDEN, 1962). Sections at 6 μ were stained with Mayer's haemalum/aqueous eosin or Heidenhain's iron haematoxylin/light green. Baker's formol-calcium fixed crops were incised, flattened onto slides with the inner or outer surface exposed and stained with Sudan Black B (PEARSE, 1968) for lipids. Chloroform-treated preparations served as controls. Bielschowsky's ammoniacal silver staining (CULLING, 1963) was also used in some preparations. Whole mounts of the formol-calcium fixed proventriculus and crop were similarly stained. Observations on the living structures isolated in 0.05, 0.1 or 0.5% methylene blue in insect Ringer (BODENSTEIN, 1946) or in the latter alone, were also made under a phase-contrast microscope.

Crops from the flies were excised and fixed for 2 h in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) at 0°C. The tissues were washed overnight in three changes of 5% saccarose in 0.2 M of the same buffer at 0°C and then post-fixed for 2 h in 0.2 M cacodylate-buffered 2% OsO_4 at pH 7.2. After fixation the material was gradually dehydrated in acetone and propylene oxide, and embedded in Epon (LUFT, 1960). Sections were cut at 600–900 nm on a Reichert OmU_2 or

LKB Ultrotome I. They were double-stained in 5% aqueous uranyl acetate (10 min) followed by REYNOLDS' (1963) lead citrate (3 min) under nitrogen gas. Observations were made with a Philips EM 300 electron microscope operated at 80 kV. Electron micrographs were taken at magnifications from 9,840 to 22,200 diameters with Gevaert 19D 50P roll film.

Radiographic studies of the flies were made using a Sterling Dental X-ray unit. The unit was adapted to give an energy output of 25 kV. The modification consisted of inserting a Variac (Claud Lyon's Ltd) autotransformer into the lead supplying the primary winding of the high tension transformer. Optimum settings were found to be: Variac output, 100 V; tube current, 7.5 mA; target to film distance, 20 cm and exposure time, 10 sec. Kodak dental X-ray film (type 48-50) was used and developed in Kodak D19b for 4 min at 26°C. It was found that the hypothetical X-ray source was too large to permit magnification between the fly and the film; consequently, the flies were narcotised singly with CO_2 and directly placed on the plastic cover of the film. They came to activity within 15 sec after being narcotised.

All weighings were made on an analytical balance, Mettler M5 (accuracy $\pm$ 0.002 mg). The flies were weighed in 3.5 x 1.5 cm plastic tubes. Isolated blood meals were weighed on 22 x 22 mm. No. 1 cover glasses. The methods specific to particular experiments are described under the appropriate sections.

Experiments and Results

A. Structure

The disposition of parts and structure of the proventriculus with its associated ducts and crop are well shown in Plates 1–4. The proventriculus forms a sort of a three-way coupling between the oesophagus, crop-duct and midgut (Plate 1). The oesophagus opens into the proventriculus as a dorsal foramen and then continues posteriorly as a slender duct to the abdomen where it widens and ends in a large crop-sac. The proventricular sinus opens dorsally into the midgut. In addition to an oesophageal sphincter behind the pharynx (JOBLING, 1932), there are three other sphincters: one in the proventriculus proper and the other two in its neighbourhood, all of which, as will be seen later, are involved in regulating the early course of blood meal. The fore-gut and midgut are separated by a strongly developed proventricular sphincter (Plate 1C). The muscularis of the oesophagus forms the posterior oesophageal sphincter near the junction of the proventriculus (Plate 1B) and that of the crop-duct forms a sphincter at its origin (Plate 1D). The large epithelial cells of the annular pad secrete vescicles of fluid. The invaginated portion of the midgut presses and rolls this fluid posteriorly into the thin membranous peritrophic tube. These observations agree with WIGGLESWORTH's (1929) description of the formation of the peritrophic membrane.

The crop wall is lined by chitinous intima, secreted and supported by a layer of flattened cells (Plate 3A & B). Overlying the intima is a

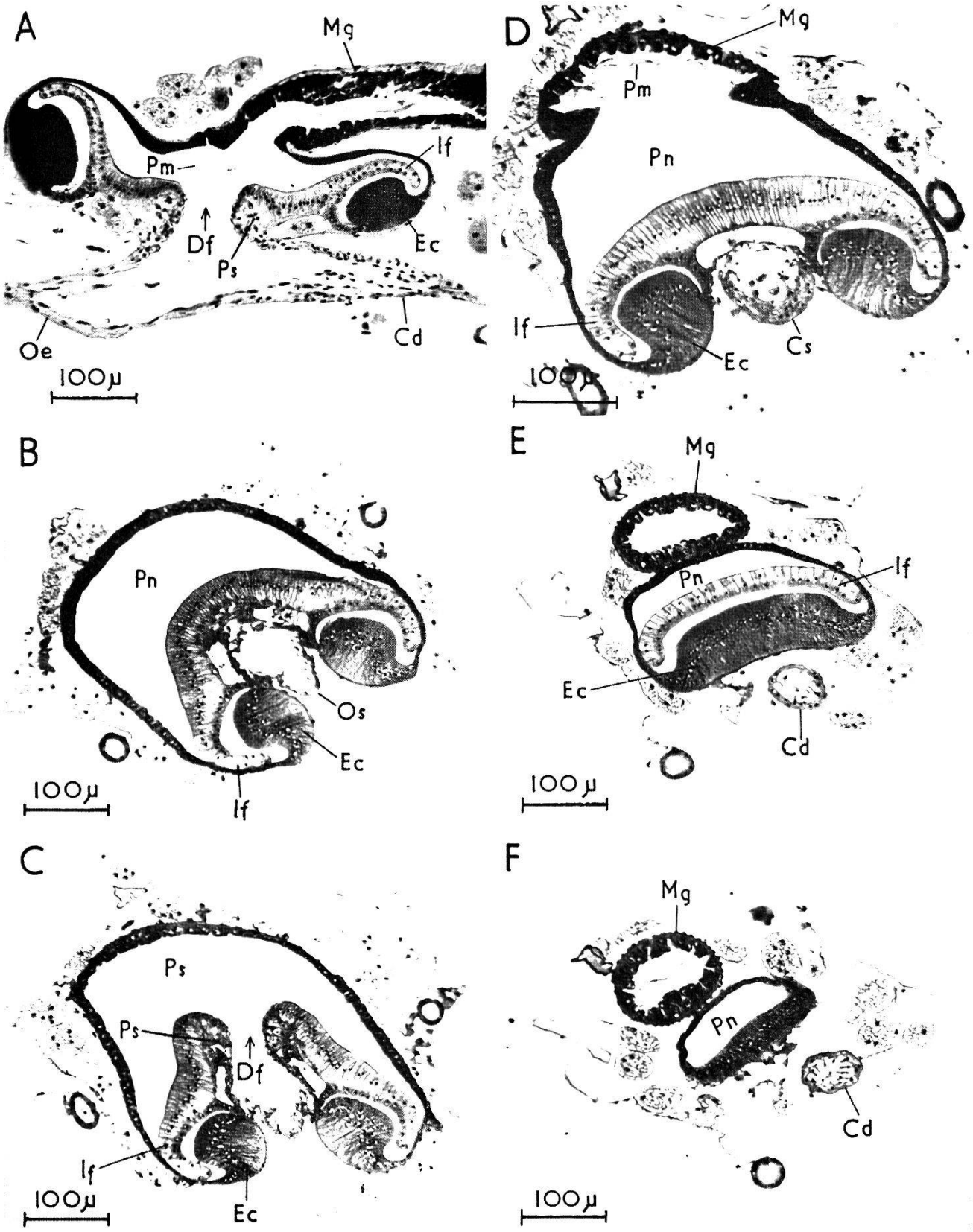


Plate I

Structure of the proventriculus. A: saggital section and B–F: transverse sections at different levels, from anterior to posterior regions. (Cd = crop-duct; Cs = crop-duct sphincter; Df = dorsal foramen; Ec = ring of epithelial cells; If = invaginated portion of fore-gut; Mg = mid-gut; Oe = oesophagus; Os = oesophageal sphincter; Pm = peritrophic membrane; Pn = proventricular sinus; and, Ps = proventricular sphincter.)

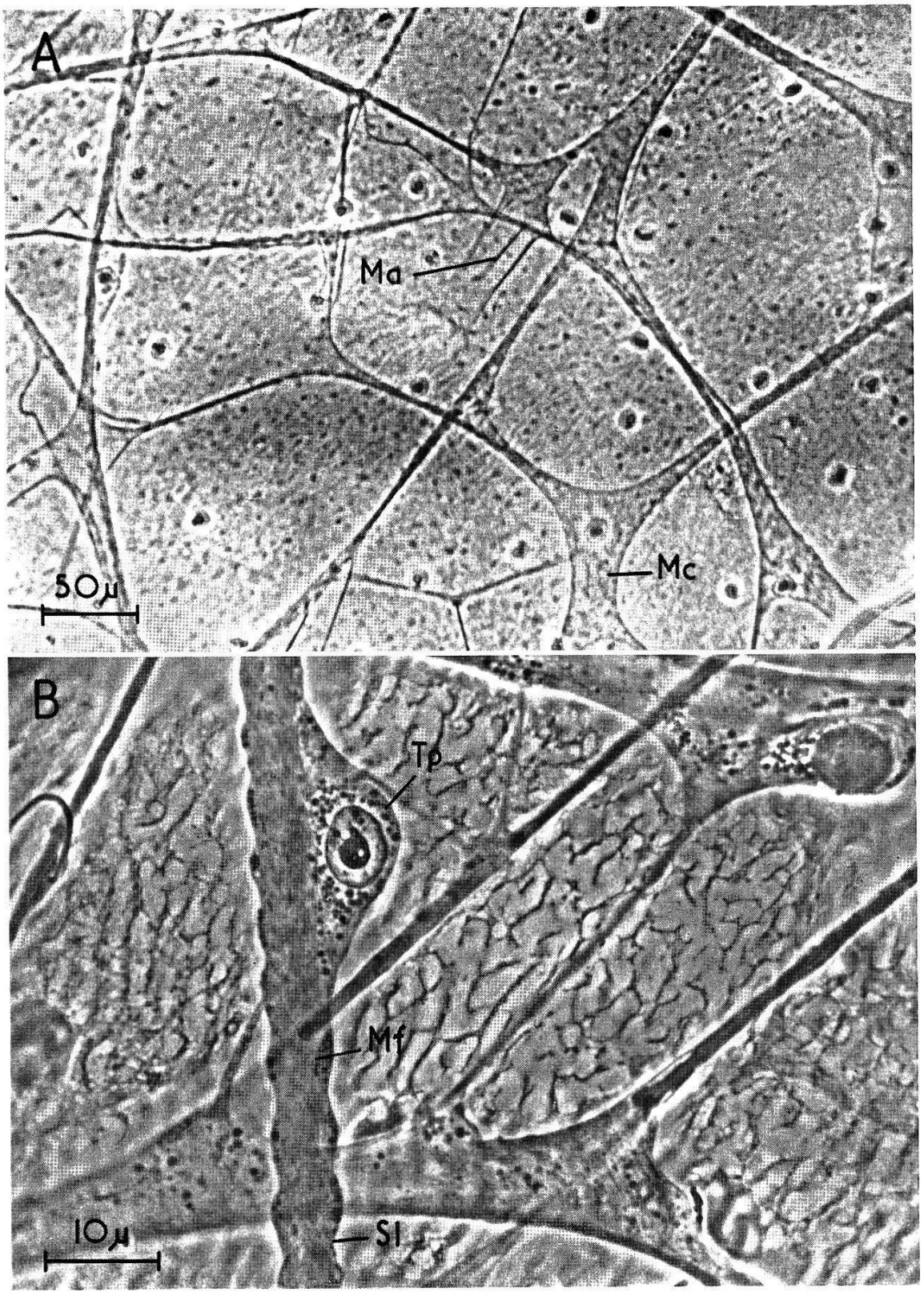


Plate II

Structure of the living crop.
A. Showing general arrangement of the muscle network.
B. Showing muscle fibre with a terminal plate.

(Lc = lateral fibrillar communication; Ma = muscle attachments to the intima; Mb = muscle cross-bands; Mc = muscle cell; Mf = myofibrils; Nc = nucleus of the epithelial cell; Sl = sarcolemma; and, Tp = terminal plate.)

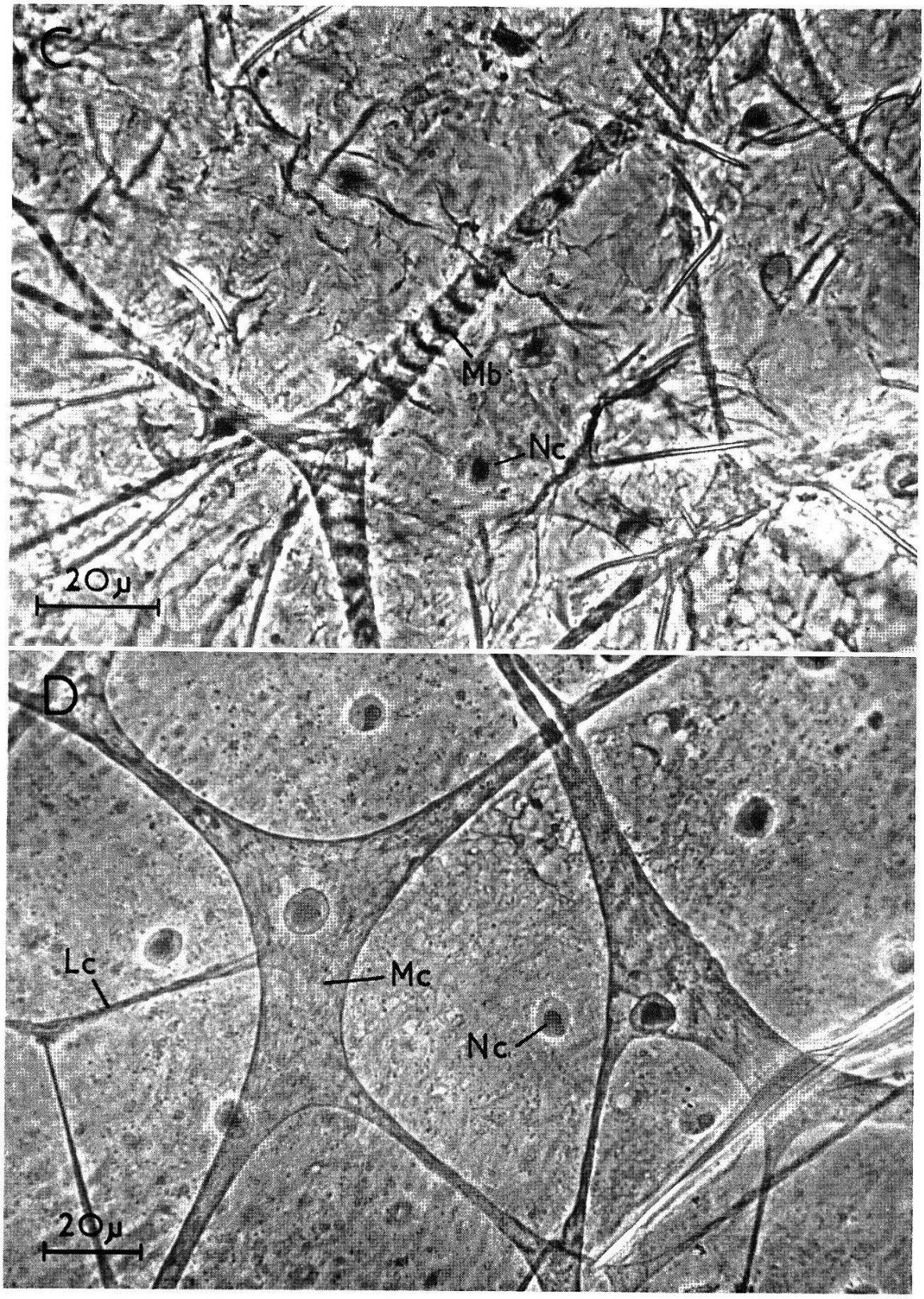


Plate II

C. Showing muscle cross-bands.
D. Showing muscle cells and lateral fibrillar communications.

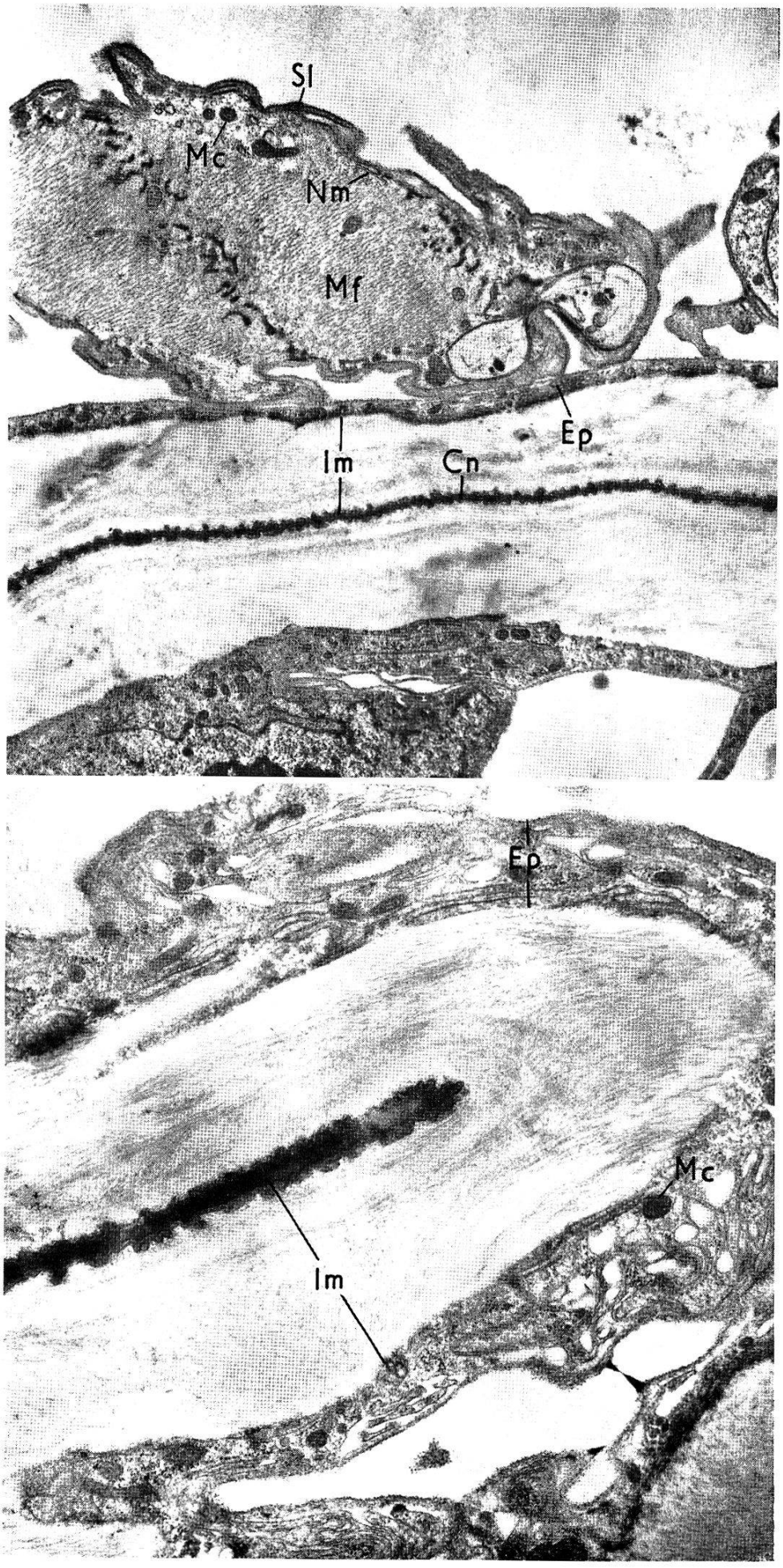
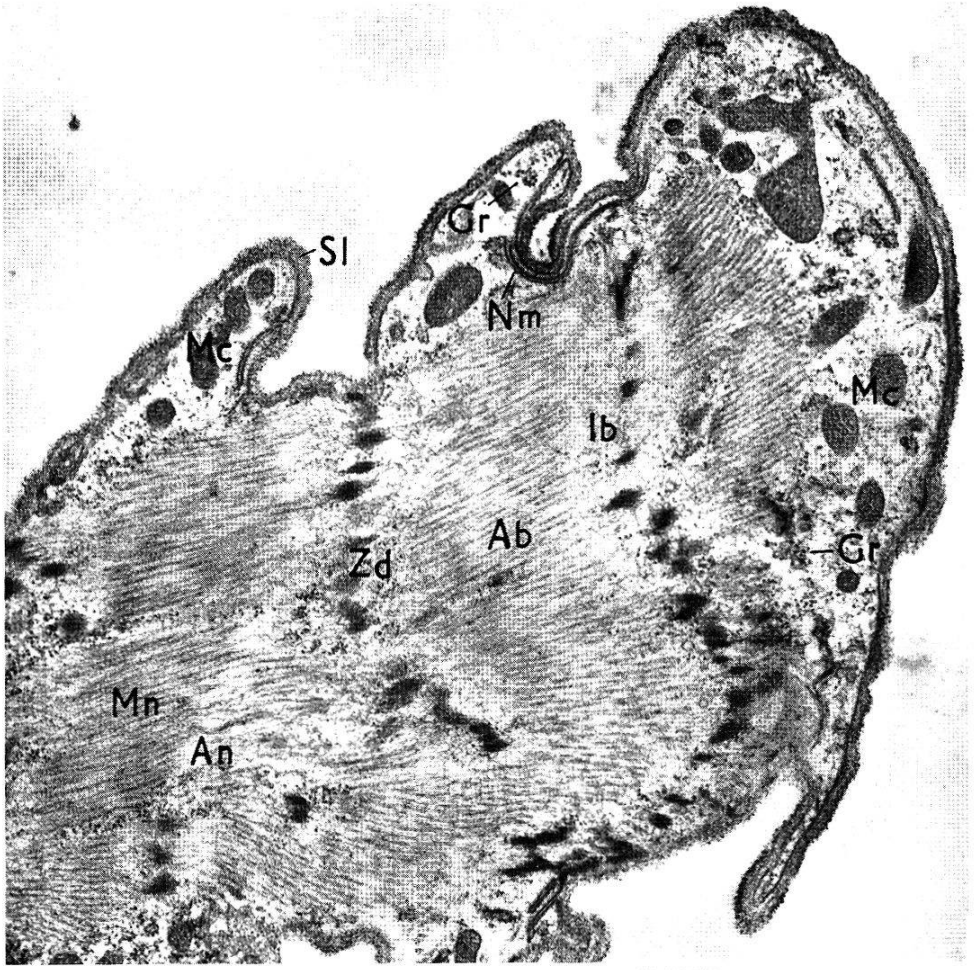


Plate III

A. Low power electron micrograph of the crop wall. 9,840×.
B. Section of the crop epithelium and cuticular intima. 17,460×.



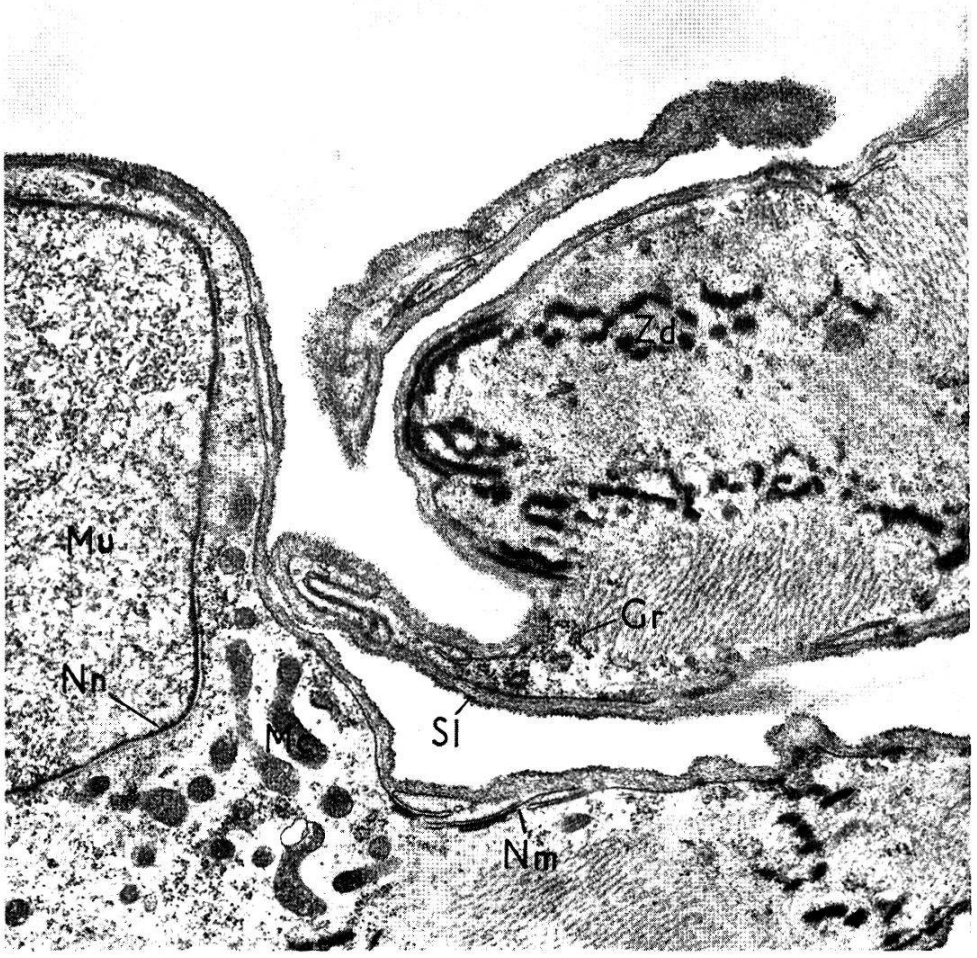


Plate IV

narrow lipoid layer. The intrinsic visceral muscles of the crop consist of flat, narrow bands which are continuations from the crop-duct. They break into a network of branching and anastomosing fibres radiating from large muscle cells (Plate 2D). The muscularis is ensheathed in a continuous and close-fitting membranous investment. Large terminal plates with granular cytoplasm are sporadically found abutting the fibres and ensheathed in a common sarcolemma. The muscle bands frequently anastomose with their neighbours by fibrillar communications which are attached at places to the cuticular intima (Plate 2A). The crop wall is supplied with a system of trachea and tracheoles. A pair of lateral oesophageal nerves described by LANGLEY (1965) run on either side of the crop duct and end in sensory cells close to the crop.

The contractile material of the muscles is composed of numerous distinct myofibrils which are divided into anisotropic A-bands and isotropic I-bands (Plate 4A). The thick myofilaments of the A-band contain myosin while the thin myofilaments of the I-band are composed of actin (KOMINZ *et al.*, 1962). Each I-band is crossed by a perforated Z-disk which divides the myofibrils into sarcomeres (Plate 4A & B).

The perforated Z-disks allow supercontraction of the myofibrils since the sarcomeres are not strictly isolated contractile units (OSBORNE, 1967; RICE, 1970). The sarcoplasm between the sarcolemma and anisotropic A-bands are packed with mitochondria and also contain nerve membrane (Plate 4) and rosettes of dark staining granules which are possibly glycogen deposits.

B. Size of the blood meal

To determine the size of the blood meal ingested and the relative amount diverted to the crop, wild 'hungry' males were weighed singly and then allowed to feed on the rabbit ears or a cow's flank. As soon as the fly had retracted its proboscis, it was chloroformed and ligatured at the thoracic/abdominal connection in order to inhibit the passage of blood from the crop to the midgut; and the fly reweighed. The ligatured fly was dissected and the crop together with its duct carefully removed. The contained blood was drained onto a cover glass and weighed. The

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Plate IV

A. Section of the crop muscle fibre showing actin and myosin fibrils. 17,460×.
B. Section of the crop wall; note perforated Z-disks and nerve membrane. 17,460×.
(Ab = A-band; An = thin actin fibrils; Cn = cuticulin containing lipoid material; Ep = crop epithelium; Gr = glycogen deposits; Ib = I-band; Im = cuticular intima; Mc = mitochondria; Mn = thick myosin fibrils; Mu = nucleus of the muscle cell; Nn = nuclear membrane; Sl = sarcolemma with close-fitting membranous investment; and, Zd = Z-disk.)

FLY	NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	MEAN	RANGE
RABBIT	wt. of unfed fly (mg.)	49.95	63.30	77.04	59.01	67.52	43.92	57.13	60.10	57.61	52.73	64.83	50.50	40.58	56.77	57.56	57.23	40.58 to 77.04
	wt. of blood ingested (mg.)	89.03	122.01	130.34	85.25	106.84	66.56	111.25	122.10	117.90	65.13	126.84	105.71	133.60	146.70	79.85	107.27	65.13 to 146.70
	Amount diverted to the crop (mg.)	48.85	81.94	85.14	56.81	74.56	43.88	71.05	86.25	86.98	41.08	72.60	65.81	109.88	115.87	46.98	72.51	41.08 to 115.87
	% of whole meal passed to the crop	54.9	67.2	65.3	66.6	69.8	65.9	63.9	70.6	73.8	63.1	57.2	62.2	82.3	79.0	58.8	66.7	54.9 to 82.3
COW	wt. of unfed fly (mg.)	56.26	53.02	53.43	66.22	60.38	49.69	58.11	61.75	53.44	55.90	—	—	—	—	—	56.82	49.69 to 66.22
	wt. of blood ingested (mg.)	124.12	100.14	138.85	156.85	99.76	89.13	110.32	102.83	120.35	145.43	—	—	—	—	—	118.78	89.13 to 156.85
	Amount diverted to the crop (mg.)	86.51	71.17	76.10	111.37	73.54	54.62	65.19	63.07	82.94	94.45	—	—	—	—	—	77.90	63.07 to 111.37
	% of whole meal passed to the crop	69.7	71.2	54.8	71.0	73.7	61.3	59.1	61.4	68.9	65.0	—	—	—	—	—	65.6	54.8 to 73.7

TABLE 1
Weight of the meal and the amount diverted to the crop

results (Table 1) indicate that the size of the ingested blood meal varied between 65.13 and 156.85 mg (mean: 113 mg), of which between 54.8 and 82.3 per cent (mean: 66%) was diverted to the crop.

It was thought that the wide variation in the size of the meal might be due to the varying hunger state in the wild flies used. To determine the influence of starvation on the size of the blood meal, wild male flies were allowed to engorge on the rabbit ears and then starved for periods 1–5 days before their next meal. Before offering the next meal, the flies were weighed and those that fed after starvation were weighed as before; the remainder were discarded. Figure 1 shows that the weight of the flies decreased with the increasing starvation period. The size of the meal, in general, progressively increased with the increasing starvation period, but was generally smaller by the fifth day of starvation. It was observed that many of the flies starved for one or five days were reluctant to feed even after persistent trials.

Many of the flies died after the fourth day of starvation while, of those which did survive, some died during or soon after feeding.

C. Early course of the blood meal

To determine the early course of the blood meal, flies feeding on the rabbit ears were interrupted at various stages of engorgement and immediately radiographed and/or dissected. The X-ray photographs are shown in Plate 5A–G. In the unfed fly the crop is seen distended

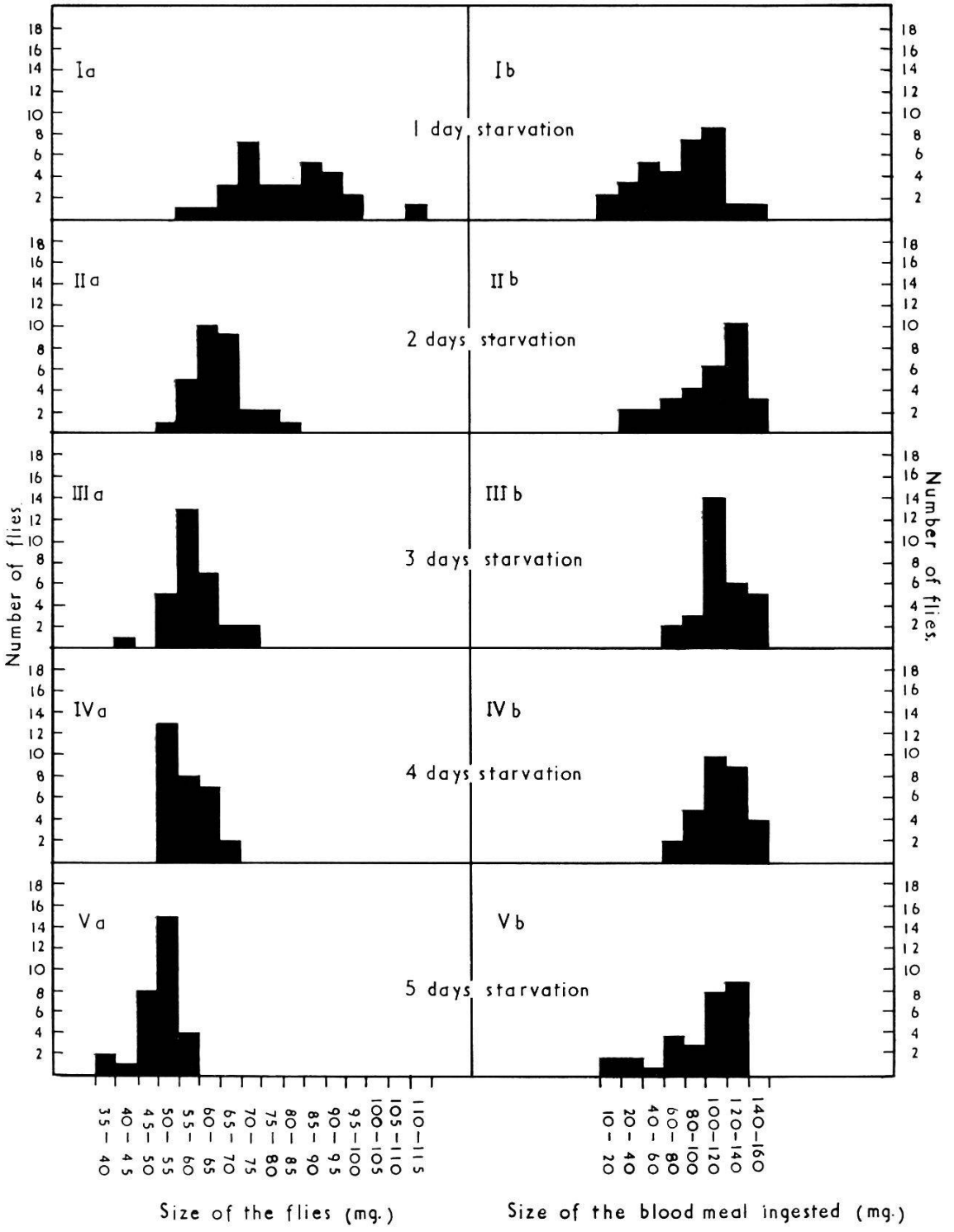

Fig. 1. Influence of starvation on the size of the flies and blood meal ingested. (30 flies were used for each day of starvation.)

with air, and as the sac is filled, the 'air bubble' shrinks. At the initial period of feeding, the blood passes into the midgut, followed by the filling of the crop.

It was also observed that the rectum of the flies whose feeding was interrupted often contained a clear fluid. It would appear that the elimination of at least water, at primary excretion, begins very early during feeding. Also during the initial period of ingestion, the abdomen

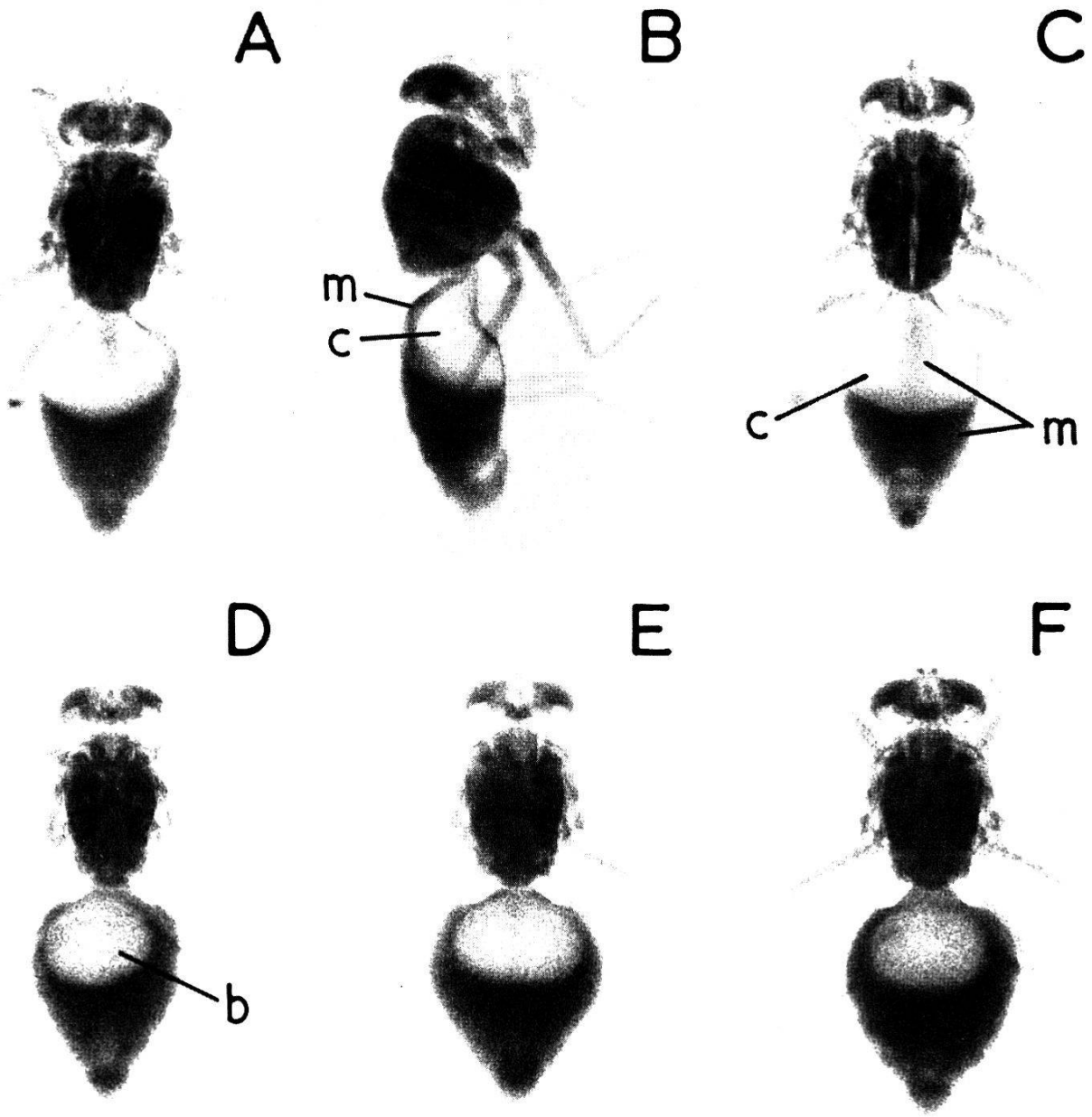

Plate V

X-ray photographs of *G. brevipalpis* at different stages of engorgement. A & B: unfed fly; C–F: interrupted in its feeding after varying intervals; G: fully fed;

distends very slowly; but after a short time, the progressive distension increases rapidly. This latter period coincides with the filling of the crop. Observations at this stage also showed that the blood meal was usually diverted to the crop before the midgut became fully distended.

D. Crop emptying

Direct observations on the proventricular region were made by cutting a window on the side of the thorax of fully engorged flies and

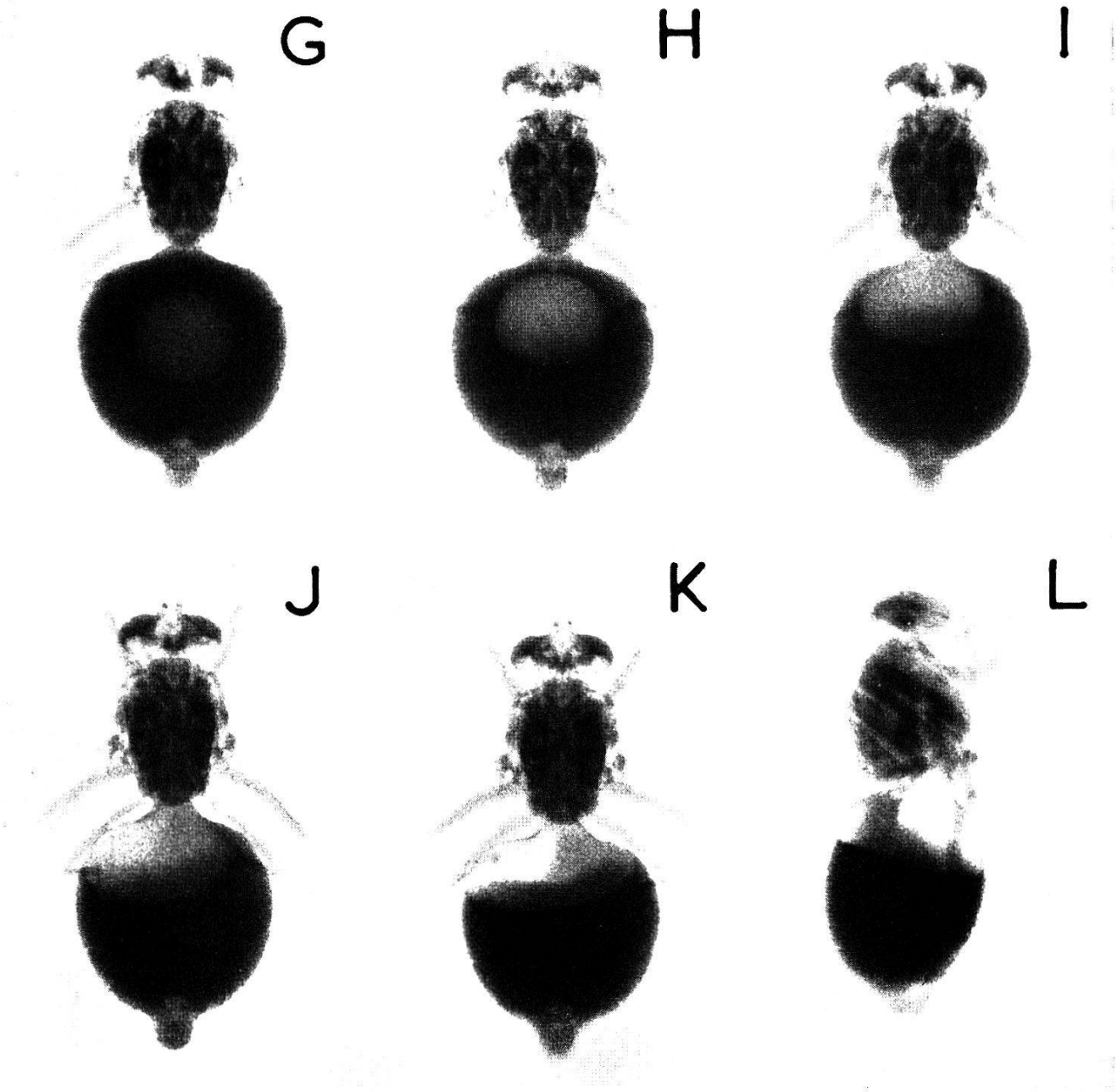

Plate V

H–K: 5 minute intervals after feeding; and, L: lateral view of K. (b = air bubble in the crop; c = crop; m = mid-gut.)

removing the tissues covering the proventriculus and its associated ducts. The *in situ* preparations were kept moist with drops of insect Ringer and observed under a 40× binocular microscope.

The crop-duct is contractile and capable of considerable distension. Its vigorous pulsations pushed the blood forwards and backwards under the proventricular portion of the oesophagus. At frequent intervals, as the proventricular sphincter relaxed, a simultaneous contraction of the oesophagus pushed the blood into the midgut with great rapidity. Strong continual peristaltic waves of the anterior segment of the midgut

squeezed the meal backwards. At times, the posterior oesophageal sphincter remained contracted inhibiting the forward movement of the blood, but the passage of the meal into the midgut was maintained by the activity of the crop-duct. Occasionally, the crop-duct sphincter remained contracted stopping the crop emptying process for a short time.

To investigate whether the sternal pressure was involved in the crop emptying process, 40 flies were singly fed on the rabbit ears and then narcotised with CO_2. Each fly was immediately dismembered and placed on its back in a perspex block fly-mould. In half the flies, the sternum adjacent to the crop was carefully pulled away, thus releasing the crop which rapidly ballooned out. The crop was kept moist with a drop of insect Ringer. In the remainder of the flies, the sternum was left intact. These served as controls.

It was observed that the experimental flies failed to empty their crops even after five hours while the controls emptied within half an hour. Also, the rate of elimination of the clear urine in the experimental flies was markedly slower and the volume of urine excreted was very small compared to the controls which periodically voided large amounts of clear excreta.

E. Rate of crop emptying

The rate of crop emptying was studied using wild male flies which were allowed to engorge on the rabbit ears and radiographed immediately, and thereafter at five minute intervals. The gradual emptying of the crop during a twenty minute period is shown in Plate 5H–L. As the blood is drained into the midgut, the air-bubble in the crop gradually increases in size until it fills the whole crop, when the latter is completely empty.

To investigate the influence of temperature on the rate of crop emptying, wild males were allowed to feed singly as before, and kept at 9°C, 25°C or 37°C for varying periods of time, after which they were dissected and the crops examined. Since it was not possible to maintain the relative humidity constant at the above temperatures, the influence of the humidity on the crop emptying rate was also investigated. Flies were allowed to engorge as before and then, one batch placed in a large polythene bag containing $CaCl_2$ and supported onto a steel-rod skeleton, with a 15% R.H. (± 5%). The other batch of flies was maintained at 80% R.H. The experiment was carried out in the insectarium at 25°C. Comparative rates of crop emptying in the wild female flies and the laboratory reared males at 25°C and 80% R.H. were also investigated.

Figure 2 indicates that the rate of crop emptying is influenced by temperature. Also, it was observed that at 9°C the rate of diuresis was

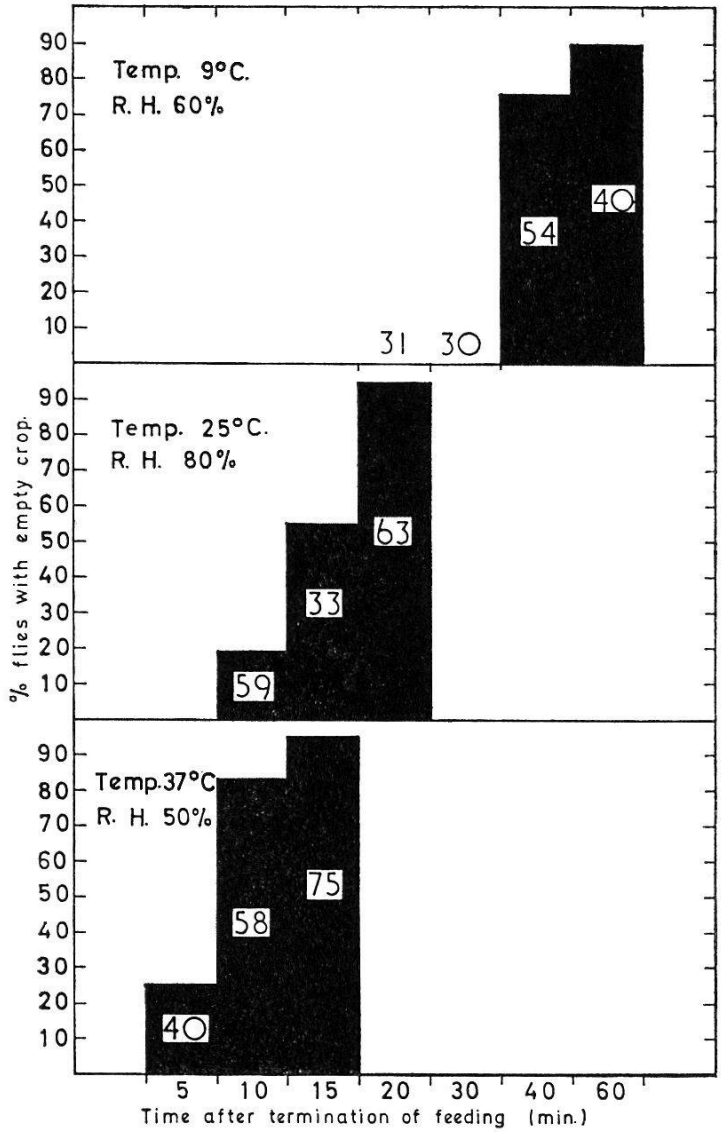

Fig. 2. Rate of crop emptying in wild *G. brevipalpis* (♂♂) at three different temperature. (The figures within the histograms indicate the numbers of flies examined.)

markedly low as compared to the higher temperatures. The results given in Figure 3 show that, (i) the relative humidity has negligible influence on the rate of crop emptying; and, (ii) the laboratory reared males and wild female flies emptied their crops earlier as compared to the wild males. For example, 97% and 87% of the laboratory reared males and wild female flies, respectively, emptied within fifteen minutes after feeding as against 55% for the wild male flies during the same period.

F. Relation of primary excretion to crop emptying

To investigate whether the crop emptying process is related to primary excretion, the rate of diuresis after the termination of feeding was determined. Wild male flies were starved for 48 hours, weighed and then fed singly on the rabbit ears.

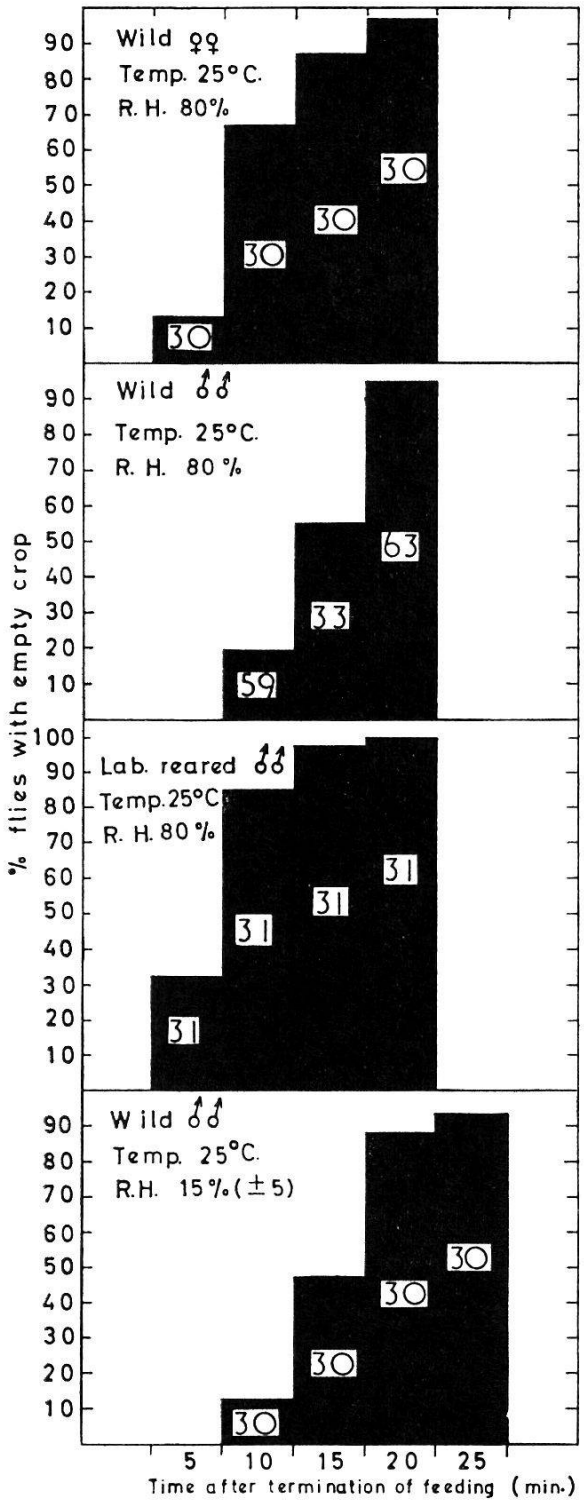

Fig. 3. Rate of crop emptying in wild and laboratory reared *G. brevipalpis.* (Figures within histograms indicate the numbers of flies examined.)

They were reweighed immediately and thereafter at five minute intervals for one hour. The flies were transferred into clean plastic tubes before each weighing. The loss in weight was taken as the amount of urine excreted. For comparison, similar investigation was carried out on the laboratory reared male flies. Evidently, the procedure is subject to at least two systematic errors: (i) loss of water by transpira-

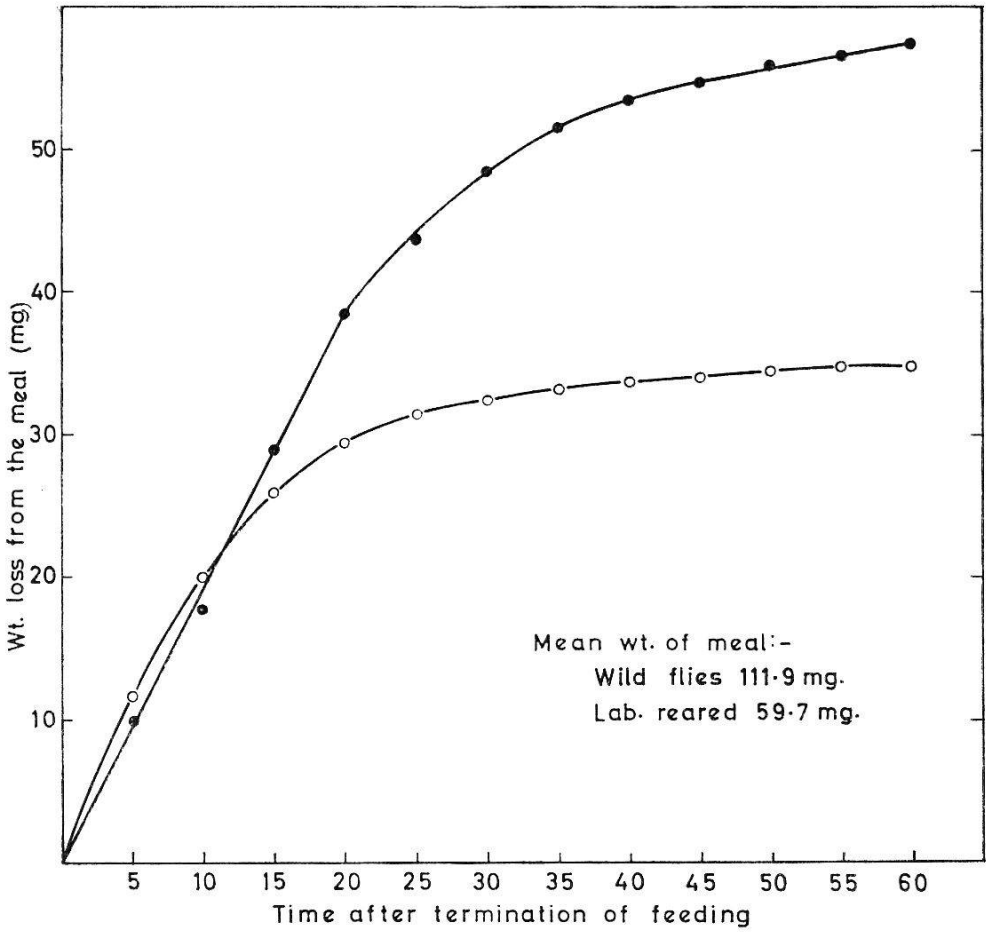

Fig. 4. Amount of urine excreted after feeding by wild (●) and laboratory reared (○) *G. brevipalpis* ♂♂.

tion; and, (ii) weight loss due to fat metabolism. However, under the present experimental conditions, these errors would be too low to be of any significance (BURSELL, 1960).

Figure 4 indicates that the wild flies eliminated an average of 49.5 mg of urine within a period of 30 minutes after feeding. This is 44% of the whole meal. As noted in (E) above, this is the period when the crop is emptied. Also, within the same period, the laboratory reared flies eliminated only 32.5 mg of urine. However, the size of the meal ingested by the two groups of flies was significantly different; average of 111.9 mg by the wild as against 59.7 mg of blood by the laboratory reared flies. This would explain the difference in the amount of urine excreted due to diuresis. Figure 4 also shows that the rate of diuresis was significantly the same for both the groups of flies.

The results given in Figure 5 show the relation of diuresis to the size of the meal in the wild and laboratory reared male flies. Although the starvation period for both the groups was the same, compared to the wild flies, the laboratory reared flies ingested significantly smaller meals. Also, irrespective of the size of the meal, the wild flies eliminated an average of 44% while the laboratory reared ones, 54% of urine due

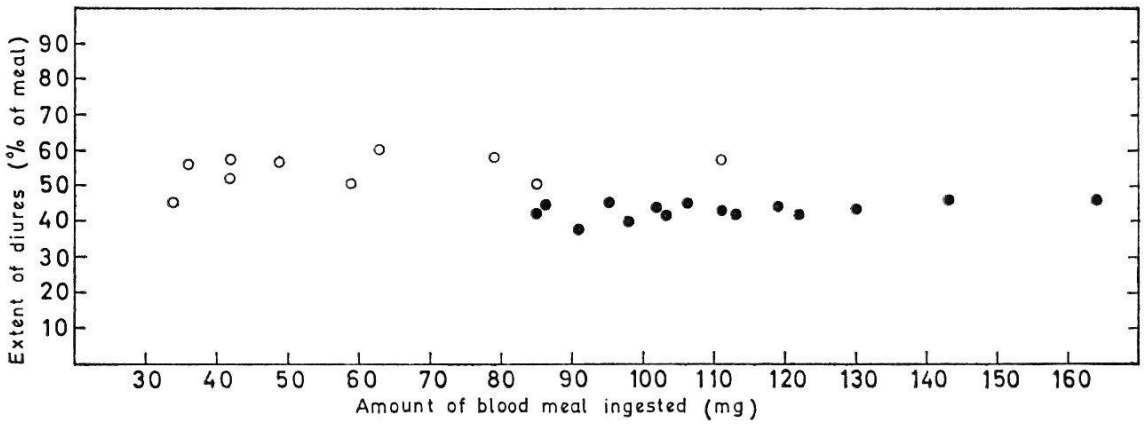

Fig. 5. The relation of the extent of diuresis 30 min after feeding to the size of the meal in wild (●) and laboratory reared (○) *G. brevipalpis* ♂♂.

to diuresis. However, since the size of the meal ingested by the two groups differed significantly, the actual amount of primary excreta eliminated by the wild flies was markedly more as compared to the laboratory reared flies.

G. The relationship of crop wall to water absorption

To investigate whether water from the blood meal is absorbed by the crop wall as presumed by GLASGOW (1963), flies were allowed to complete ingestion on the cow's flank or rabbit ears, and then one of the following two procedures were adopted: (a) Individual crops of the engorged flies were isolated immediately or 10 minutes after feeding, the blood poured onto cover glass and weighed. The blood was then dried over phosphorus pentoxide and reweighed. The water content was obtained by subtraction. (b) The engorged flies were immediately ligatured at the thoracic/abdominal connection to inhibit the passage

Series	Number of flies	Host animal	Time after feeding (min)	Mean Water Content of blood meal in the crop (%)	S. E.
1a	10	Cow	0	85·05	0·04
1b	10	"	10	83·43	0·86
1c*	10	"	15	84·75	0·07
2a*	15	Rabbit	0	79·48	0·21
2b*	12	"	30	79·72	0·20

* Flies were ligatured at the thoracic/abdominal connection.

TABLE 2

Water content of blood meal in the crop

of blood into the midgut; the blood water content was similarly determined immediately, 15 min or 40 min after feeding.

Results in Table 2 show that the water content of the blood meal remains unchanged for at least 40 min, which is long enough for the crop to empty under normal conditions.

Discussion

Amongst the blood sucking insects, *Glossina* species are remarkable for their ability to ingest enormous meals in a very short time. In the present study, the largest meal taken by a wild male *G. brevipalpis* was 164.3 mg, increasing the weight of the fly by 269%. The blood is sucked by the action of the pharyngeal pump aided by waves of peristalsis passing positeriorly in the oesophagus. At feeding, 55–82% of the whole meal was diverted to the crop, distending the latter enormously. The immense distension with blood followed by shrinking during crop emptying is made possible by the characteristic super-contractile muscle network with its numerous mitochondria and rosettes of glycogen deposits. In many cases, the smaller flies imbibed larger meals compared to the heavier flies. It is conceivable that the size of the meal is dependent on the physiological state of the fly, such as, the hunger stage and the degree of tissue dehydration.

The weight of the fly progressively decreased while the meal size increased with the increasing starvation period. Subsequent to feeding, the loss in weight is due to diuresis, metabolism of various substrates, excretion and water loss through transpiration. As the starvation period is increased, the nutrient reserves would become gradually exhausted and the tissues increasingly dehydrated. In order to replenish the nutrients and bring the tissues to full hydration, the fly must ingest larger meals as the interval between the feeds becomes greater. This applies up to a certain limit. For example, after the fourth day of starvation, many flies died, while of those that survived and fed, many sucked up smaller meals or died during or soon after feeding apparently through exhaustion. The present study also demonstrates that some flies can survive for five days without a feed and still manage to suck enormous meals. The flies, however, were brought from the field and consequently handled many times before they were finally brought to the laboratory. Such disturbance and the artificial conditions under which they were maintained may have somewhat affected them. JACKSON (1937, 1949), showed that in the field, *G. swynnertoni* and *G. morsitans* can survive for 10–12 days without food. It is conceivable that in its natural environment, the present species also can withstand longer period of starvation than the current study indicates.

The presence of air in the crop of the unfed fly helps to maintain a positive pressure, preventing the organ from collapsing. The air space in the crop decreases on ingestion followed by an increase as the meal is released into the midgut. The air is drawn into the crop not long after emergence, but the emptying and filling with air at ingestion and crop emptying, respectively, has not been determined. It is conceivable that the air in the crop is compressed during filling and subsequently decompressed as the sac is emptied. The present results clearly demonstrate that the meal is first passed into the midgut. Soon, however, it is diverted to the crop. Regurgitation of the blood is prevented by the anterior oesophageal sphincter.

At the initial period of feeding the abdomen swells very slowly, but, after a short time, the distension continues at a remarkable speed. The latter period is when the crop is filled. The passage of the blood into the sac begins even before the midgut is filled to capacity. In fact, in some cases very little was passed into the midgut when the meal was diverted to the crop. It would seem that diversion to the crop begins as soon as there is back pressure from the midgut.

The emptying of the meal from the crop into the midgut is regulated by a series of four sphincters. The anterior and, at times, the posterior oesophageal sphincters prevent regurgitation as crop emptying proceeds. The present results show that crop emptying is an active process. After engorgement, the mechanical pressure imposed on the sac by the stretched abdominal sternum, aided by the contractions of the crop wall muscle-network, push the meal forward into the crop-duct. The strong pulsations of the latter with the concurrent peristalsis of the oesophagus forces the blood into the midgut through the proventricular sphincter which opens at frequent intervals. At the final stages of the emptying process when the crop has partially shrunk, the pumping-like action of the crop-duct sucks the blood into it and then into the midgut until the sac is completely empty. Back flow from the midgut is prevented by the proventricular sphincter.

An increase in temperature causes the rate of crop emptying to increase while the rate falls in response to a drop in temperature. Also, it was observed that primary excretion is likewise sensitive to temperature changes. Drops of clear urine were voided at unusually longer intervals when the flies were placed at the lower temperature than when they were exposed to the higher temperatures. In *Periplaneta americana* (PATTON *et al.*, 1959), excretion is primarily a secretory process energised by an oxidative enzyme system. It has been suggested that this is true of insects generally (WIGGLESWORTH, 1965). In *Rhodmius prolixus* (MADDRELL, 1964), for example, a change in temperature has a marked effect on the rate of excretion. If the crop emptying rate is dependent upon primary excretion, then acceleration or retardation of the latter

process should likewise affect the rate of crop emptying. That this, in fact, is correct is evident from the present study which shows that the ambient temperature influences the rate of diuresis and indirectly the rate of meal release from the crop. Observations showed that, at the same ambient temperature, some flies voided drops of urine between unusually long intervals, indicating reduced rate of excretion. In such flies the crop was emptied at an exceedingly low rate. Those flies in which primary excretion was delayed for an abnormally long time after feeding, the blood was retained in the sac until such time when diuresis occurred. These observations lend further support to the suggestion that blood release from the crop is closely associated with primary excretion.

The blood from the crop is drained into the midgut exceedingly rapidly after the proboscis has been withdrawn. For example, at 25°C, in the majority of the flies the crop emptied within 5–20 minutes after feeding. Rapid crop emptying rate has also been observed in wild *G. pallidipes, G. fuscipes* and *G. morsitans* (unpublished observation). However, LESTER and LLOYD (1928) and WIGGLESWORTH (1929) observed that the crop contained blood some hours after a meal. It would seem that their flies were exposed to some abnormal conditions. In our laboratory, the flies feeding on defibrinated blood through rat skin membrane exhibit unusually low crop emptying rate, suggesting inefficient artificial feeding technique. This is currently being investigated.

The slightly higher rate of crop emptying in the wild females as compared to wild males is almost certainly due to the higher diuretic rate in the former. It was observed that the females, and particularly the pregnant ones, excreted urine at a remarkable rate. The laboratory reared males generally emptied their crops earlier than the wild male flies. Since the rate of diuresis in both the groups was the same (Fig. 4) and, since the laboratory reared flies ingested significantly smaller meals, diuresis and hence the crop emptying was completed earlier in these flies as compared to the wild male flies.

It was observed that in many flies the rectum became distended with clear urine during ingestion of a meal indicating that primary excretion begins sometime during feeding. The fly normally excretes drops of clear fluid at certain intervals not long after, and in some cases even during feeding. As the meal in the midgut becomes dehydrated, more is passed into it from the crop. The crop then, is, though temporary, an efficient reservoir since it provides extensible space in which the fly fills and stores enormous volume of blood until such time when certain unwanted materials, and particularly excess water, can be eliminated from the meal by the anterior midgut segment (WIGGLESWORTH, 1929) by diuresis. The present study also demonstrates that the wax-like lining of the crop wall prevents any absorption of water and that the blood water content within the crop remains unchanged.

This is also supported by the observation that the volume of urine eliminated declined appreciably when the crop was not allowed to empty for five hours compared to the normal half hour. It is clear therefore that the absorption of water at diuresis is not the function of the crop wall.

The present results also demonstrate that excretion of at least water proceeds at an exceedingly high rate after feeding. For example, within 30 minutes after termination of feeding, the fly lost its weight by an average of 38% through diuresis. The elimination of a very large amount of water at such a remarkable rate would demand a high energy consumption. However, to offset this is the advantage that the tissues are thus exposed to the hypotonic hemolymph for a brief period only and that the fly is able to unload its great weight very quickly.

Immediately after feeding the fly is so heavy that according to GLASGOW (1961) the speed of *G. swynnertoni* is reduced from about fifteen to only three or four miles per hour. Clearly rapid weight loss after feeding is of immense advantage to the fly.

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