

THE RELATIONSHIPS BETWEEN NUTRITION, HORMONES
AND REPRODUCTION IN THE BLOWFLY *CALLIPHORA*
ERYTHROCEPHALA (MEIG.)

II. THE EFFECT OF REMOVING THE OVARIES, THE CORPUS ALLATUM
AND THE MEDIAN NEUROSECRETORY CELLS UPON SELECTIVE FEEDING,
AND THE DEMONSTRATION OF THE CORPUS ALLATUM CYCLE

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INTRODUCTION

Calliphora females select 'protein' (Marmite in milk) and carbohydrate (sugar in water) solutions in proportions which vary with the different phases of the reproductive cycle (Strangways-Dixon, 1959, 1961). Since non-reproducing females (fed on sugar solution but no 'protein') show no signs of cyclical feeding, it seemed possible that selective feeding was a response to the cyclical requirements of the developing ovaries. Since ovarian development in *Calliphora* is controlled by the corpus allatum (c.a.) and the median neurosecretory cells (m.n.c.) of the brain (E. Thomsen, 1940, 1942, 1948, 1952) it seemed possible that selective feeding was also influenced by these secretory organs. The effects of extirpating the ovaries, the m.n.c. and the c.a. upon selective feeding were therefore investigated.

METHODS

The three operations—ovariectomy, allatectomy and removal of the m.n.c.—were performed as described by E. Thomsen (1942, 1952) with the following modifications. Loops of fuse wire instead of plasticene strips were used to hold the insects in position and (in allatectomy and m.n.c. removal) to prise the heads away from their bodies. During ovariectomy operations, the positions of the ovaries varied slightly, making it difficult to be certain that the probing forceps had grappled the ovaries rather than the convoluted gut. In the latter case, the gut was often torn and the fly had to be discarded. Therefore, instead of grappling directly for the ovaries, use was made of the rich tracheal supply which derives from the spiracles situated on the fourth and fifth abdominal segments. A small tear was made just posterior to the spiracle on the fifth abdominal segment, the forceps were inserted through the opening and grappled the tracheae as they emerged from the spiracle. The tracheae were pulled out 'hand over hand' using two pairs of forceps until the ovaries themselves emerged from the slit. The gonads were held with one pair of forceps whilst the tracheae and oviduct were cut with the other pair. The cut ends were pushed back into the abdomen and the wound was sealed with 60° C. wax. The operation was then repeated for the other ovary. It is important to perform this operation as soon after emergence

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as possible because after the flies have fed, the chances of perforating the gut increase.

The selective feeding technique and the method for culturing the flies has been described in a previous paper (Strangways-Dixon, 1961).

THE EFFECT OF OVARIECTOMY UPON SELECTIVE FEEDING
AND CORPORA ALLATA VOLUMES

Selective feeding

Nineteen ovariectomy and thirty-two control operations were performed on the day of emergence. After 6 days of sugar diet [females are unable to reproduce on a sugar diet (Fraenkel, 1940), so this initial 6 days in a sugar culture was to give the flies time to utilize their remaining pupal protein reserves and thus remove a variable factor which might have influenced selective feeding] fifteen ovariectomized and eight operated control females were isolated in feeding units. Ten ovariectomized and five operated control flies were given carbohydrate and 'protein' solutions whilst the remainder were given carbohydrate solution only. Selective feeding continued for 15 days during which all carbohydrate controls and three 'protein'-ingesting (ovariectomized) females died. The latter are excluded from the graph but the former are included up to the day before death. After the experiment, the flies were dissected and the c.a. and the ovaries were examined. The 'protein'-ingesting controls contained mature ovaries. The averaged ingestions of the ovariectomized females are shown in Fig. 1. Since the selective feeding of the operated controls was cyclical, averaged ingestions would conceal the individual peaks and so are avoided. For this reason the feeding of only a single female is shown (Fig. 2). In this female, as in the others, the characteristic carbohydrate cycle is still apparent.

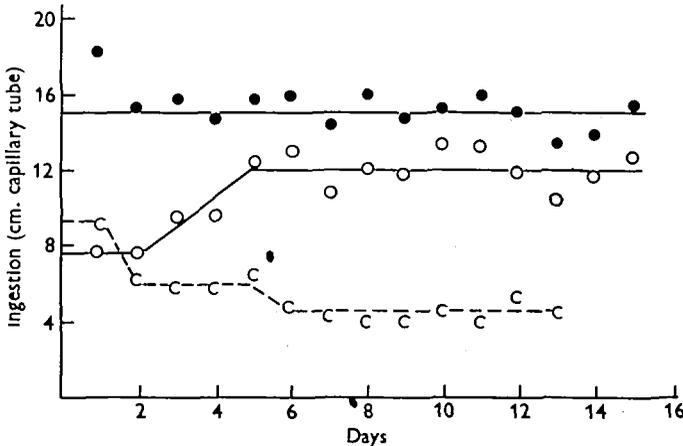


Fig. 1. The effect of ovariectomy upon selective feeding. ●, total ingestion. O, carbohydrate ingestion. C, carbohydrate ingestion of control 'sugar' flies.

Fig. 1. shows that the carbohydrate ingestion of an ovariectomized fly—provided the fly is also allowed 'protein'—increases from an initial low level but then remains high. 'Protein' intake, on the other hand, is high initially but decreases to a constantly low level, and the total ingestion remains the same throughout. This selective feeding

As the same as that of a normal reproducing female (Strangways-Dixon, 1961, Fig. 8) except that the cycle is not repeated. It is therefore concluded that *selective feeding is independent of direct ovarian control*, but that *the ovaries—probably because they utilize the ingested foods—are responsible for the succession of one cycle upon the other.*

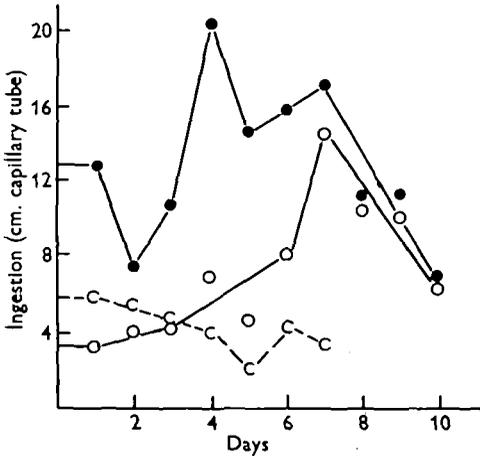


Fig. 2

Fig. 2. Operated controls for ovariectomized females. As for Fig. 1.

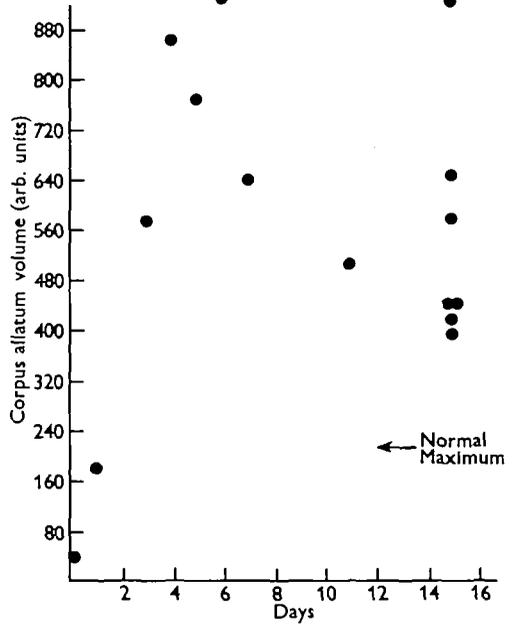


Fig. 3

Fig. 3. Corpora allata volumes of ovariectomized females.

Corpora allata volumes

A second experiment was conducted synchronously with the one described above and its purpose was to follow the volume changes of the c.a. of ovariectomized females.

Of the twenty-four females which were castrated on the day after emergence, sixteen died during the experiment. The unusually high mortality was probably due to the forceps piercing the alimentary canals which were swollen after 24 hr. feeding. The flies were maintained as a sugar culture for 6 days and then meat was added. The females were dissected during the succeeding 11 days and the c.a. volumes were plotted against the number of days after the addition of meat to the diet (Fig. 3). The points shown on the fifteenth day are those of the seven females which survived the selective feeding experiment described in the preceding section. It can be seen that the c.a. of ovariectomized flies become hypertrophied by the third day and remain thus for at least 15 days. This confirms E. Thomsen's results (1940, 1942).

THE EFFECT OF ALLATECTOMY UPON SELECTIVE FEEDING

On the day of emergence, twenty-one females were placed in feeding units and were fed carbohydrate solution only. On the following day, twelve were allatectomized and six control operations were performed. The remaining three females were left as

unoperated sugar controls. The flies were starved for 24 hr. and were then allowed to ingest carbohydrate solution for a further 4 days. At this stage, 'protein' solution was added to the diet of all operated flies and selective feeding began.

At the completion of 14 days selective feeding, eight of the allatectomized females still survived. Four of these contained mature ovaries but in the others, egg development had stopped at the yolk deposition stage. The selective ingestions of the latter were averaged and the results are shown in Fig. 4.

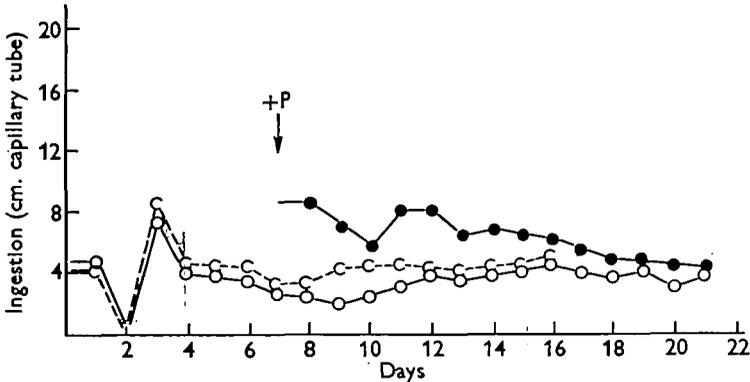


Fig. 4. The effect of allatectomy upon selective feeding. +P, 'protein' was added to the diet at this point. Otherwise as for Fig. 1.

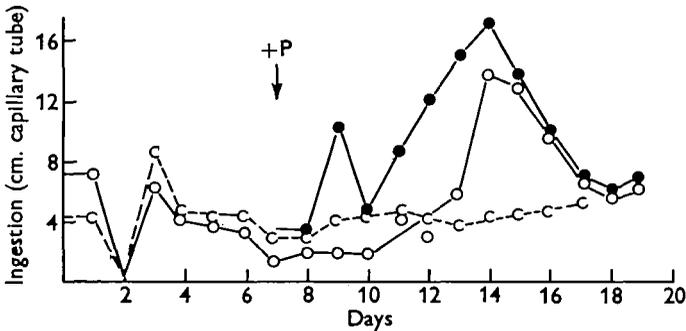


Fig. 5. Operated controls for allatectomized females. +P, 'protein' was added to the diet at this point. Otherwise as for Fig. 1.

The five surviving operated controls all contained mature ovaries. The selective feeding of one female and the averaged ingestions of the sugar controls are shown in Fig. 5. The carbohydrate ingestion of the operated control shows the normal cycle whilst the sugar control flies, as usual, consume a constantly low volume of carbohydrate.

Fig. 4. indicates that allatectomy results in carbohydrate ingestion remaining constantly low in spite of the fact that considerable quantities of 'protein' are ingested. It is therefore concluded that *the increase in carbohydrate ingestion* (which normally occurs during yolk deposition) *is dependent upon the presence and activity of the c.a.* (see Strangways-Dixon, 1959).

THE EFFECT OF OVARIECTOMY COMBINED WITH ALLATECTOMY
UPON SELECTIVE FEEDING

Ovariectomy results in the increase of carbohydrate consumption to a constantly high level (Fig. 1). Allatectomy results in carbohydrate consumption remaining constantly low (Fig. 4). If, as was thought, the normal increase in carbohydrate ingestion was dependent upon the c.a. and was independent of the ovaries, then the extirpation of both these organs should result in a selective feeding similar to that of an allatectomized female.

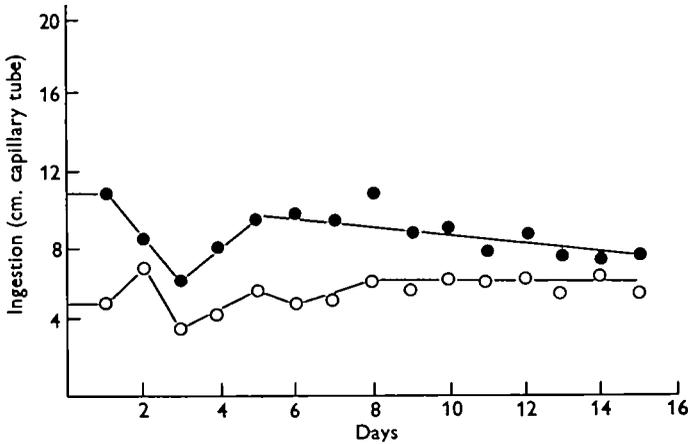


Fig. 6. The effect of ovariectomy together with allatectomy upon selective feeding. As for Fig. 1.

Twenty-four females were ovariectomized on the day of emergence and, after 6 days in a sugar culture, sixteen of the twenty-three survivors were allatectomized. This experiment was run in conjunction with those mentioned earlier in this paper and so control allatectomy operations were performed on twelve of the ovariectomy controls not in use in the other experiments. After a further 3 days in a sugar culture, the flies were isolated in feeding units. Twelve ovariectomized-allatectomized females and eight operated controls survived up to this period. Eight of the former and five of the latter were fed carbohydrate and 'protein' solutions and the remainder received carbohydrate solution only. All carbohydrate controls and one fly from the mixed diet groups died early in the experiment and so are excluded from the graphs. The selective ingestions of the double-operated females are averaged (Fig. 6), but since the ingestions of the controls were cyclical, only a single representative example is shown (Fig. 7.)

It is concluded from these results that ovariectomy combined with allatectomy results in carbohydrate consumption remaining at a constantly low level (Fig. 6). This supports the hypothesis that c.a. activity induces an increase in carbohydrate consumption.

THE CORPUS ALLATUM IN RELATION TO AGE, SIZE OF FLY
AND REPRODUCTION

Since it was thought that c.a. activity induced an increase in carbohydrate ingestion, it followed that the cyclical ingestions of reproducing females should be a result of cyclical fluctuations in the activity of the c.a. The c.a. of reproducing females were therefore examined in the hope of discovering cyclical changes in volume.

A total of 105 females were removed from a reproducing culture, normally in daily batches of five, over a period of 27 days from emergence. These were dissected and their c.a. and ovaries were measured in arbitrary units (see Strangways-Dixon, 1961). Flies caught whilst ovipositing were noted.

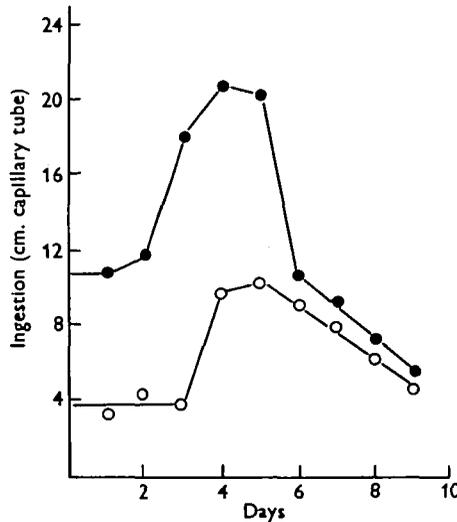


Fig. 7. Operated controls for ovariectomized allatectomized females. As for Fig. 1.

The relation of the c.a. volumes to age is shown in Fig. 8. The results confirm E. Thomsen's (1942) results in that the c.a. of mature females are larger than those of newly emerged flies. An additional observation is that the c.a. volumes taken from different flies of the same age on any single day show a very large scattering which does not seem to alter with age. Fig. 9 indicates that the c.a. volumes are not related to the lengths of the females as the scatter of the c.a. volumes is similar for each of the three lengths of fly dissected. Since there was so much variation in c.a. volumes at any given age, it was considered possible that more accurate information would be obtained if these volumes were plotted against the lengths of eggs taken from the same fly. In flies with mature eggs, however, c.a. volumes are plotted against the secondary oocytes (Fig. 10). The latter comparison is necessary because reproductive cycles tend to overlap and so the sequence of events is lost in a gravid female unless the succeeding cycle is taken into consideration.

Fig. 10 shows that the volumes of c.a. in reproducing females fluctuate cyclically: *The c.a. increase in size during the early stages of egg development and decrease in volume during yolk deposition* (see Strangways-Dixon, 1959). This supports the hypothesis that c.a. activity influences carbohydrate consumption.

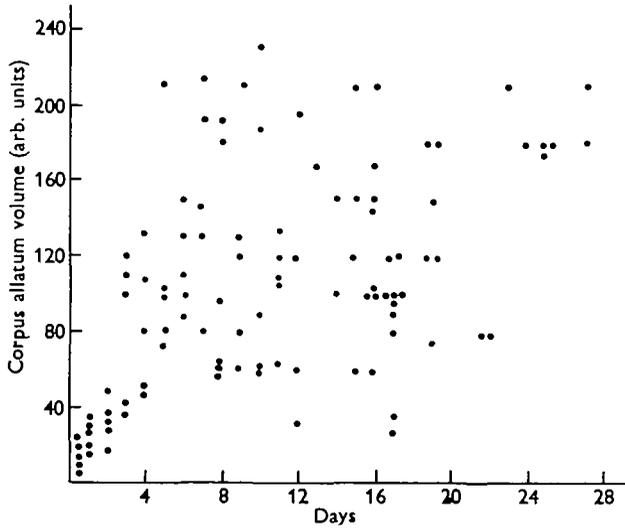


Fig. 8. Corpora allata volumes in relation to age.

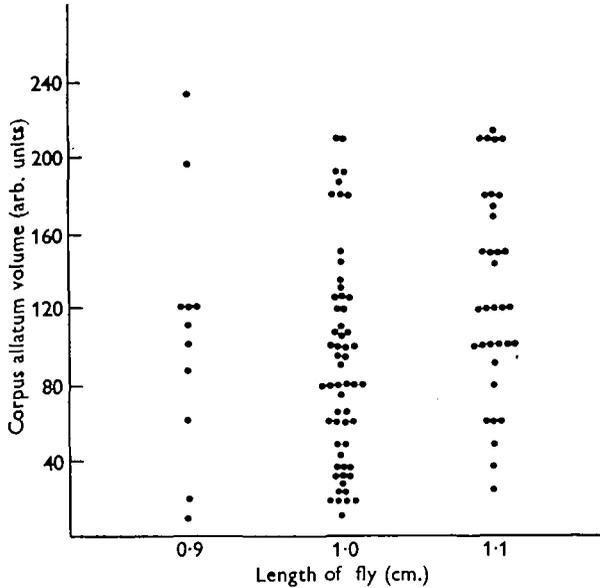


Fig. 9. Corpora allata volumes in relation to the lengths of flies dissected.

THE EFFECT OF REMOVING THE MEDIAN NEUROSECRETORY CELLS
UPON SELECTIVE FEEDING

Having investigated the influences of the ovaries and the c.a. upon selective feeding, attention was turned to a third possible controlling factor—the m.n.c. The extirpation of these cells is known to prevent egg development in *Calliphora* (E. Thomsen, 1948, 1952).

The m.n.c. were removed from forty females and control operations were performed on thirty females within 24 hr. of emergence. After 6 days of sugar diet the thirty

surviving females (lacking m.n.c.) and sixteen of the operated controls were isolated in feeding units. Twenty-four of the former and ten of the latter flies were given carbohydrate and 'protein' solutions whilst the remainder received carbohydrate solution only. Thirteen mixed diet and four carbohydrate females (lacking m.n.c.), and two mixed diet and four carbohydrate females (operated controls) died during the remainder of the experiment. The ingestions of flies without m.n.c. are averaged and the carbohydrate controls are included up to the day before death (Fig. 11). For the usual reason, only a single control fly is represented (Fig. 12) and since carbohydrate ingestion is cyclical, it is assumed that the operation without removing the m.n.c. does not appreciably influence selective feeding.

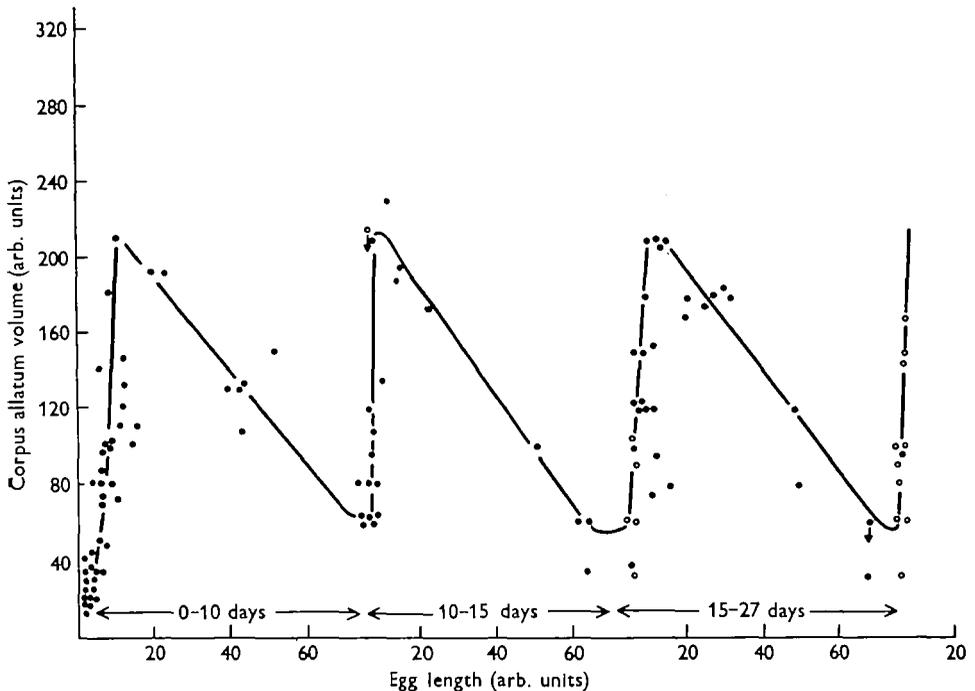


Fig. 10. Corpora allata volumes in relation to the reproductive cycle. ●, normal measurements. O, oocytes adjacent to mature eggs. ↓, these flies were ovipositing when caught for dissection.

A total of two sugar controls and seven 'protein'-ingesting flies (lacking m.n.c.) were removed from the units during the experiment. These were dissected and the c.a. were removed and measured. The volumes of the c.a. were smaller than those of reproducing females and were only slightly larger than those of sugar flies. These results confirm those of E. Thomsen (1952).

Fig. 11 indicates that flies lacking m.n.c. ingest a constantly low volume of carbohydrate. 'Protein' ingestion is high on the first day only and then declines abruptly to negligible quantities. It appears that the *removal of the m.n.c. results in the failure of females to ingest 'protein' except for an initial brief period.* The preliminary intake of 'protein' may be a consequence of some residual neurosecretion in the blood or in the corpus cardiacum. It is as well to mention at this stage that at the time of this

Experiment (April 1958) E. Thomsen was performing similar investigations (E. Thomsen & Møller, 1959) in which she found that females lacking m.n.c. continued to ingest meat normally. We have discussed this apparent contradiction and can find no answer except that possibly the attraction of meat is so strong that it overcomes the effect shown in the present experiment in which a solution of milk and Marmite was used instead of meat. Conclusions arising from the results where this contradiction is applicable must therefore be regarded as only very tentative.

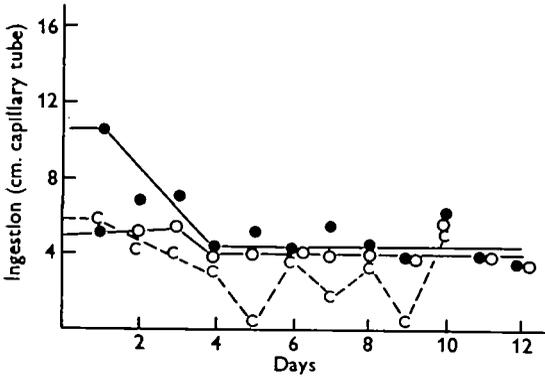


Fig. 11

Fig. 11. The effect of removing the median neurosecretory cells upon selective feeding. As for Fig. 1.

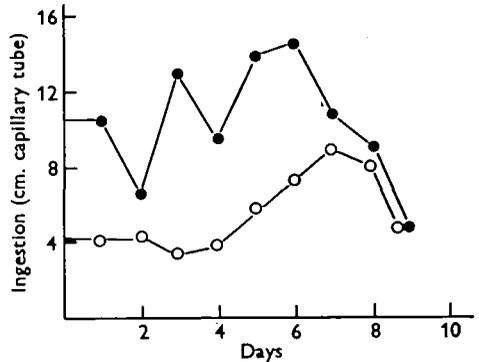


Fig. 12

Fig. 12. Operated controls for removal of the median neurosecretory cells. As for Fig. 1.

It became apparent towards the end of the experiment that if females (without m.n.c.) did not ingest 'protein', then any previous results obtained by the same operation were in fact due to the influence of the m.n.c. upon the ingestion of 'protein'. It was decided therefore to *force* the flies, which remained at the end of the experiment, to ingest 'protein' and to see whether the ovaries and the c.a. would then develop. Of the four remaining females, two were fed on carbohydrate solution and the other two on a mixture (1:1) of carbohydrate and 'protein'. The two latter flies ingested the mixture as if it were carbohydrate and were thus forced to consume the 'protein'. On dissection—after 6 days of this treatment—'protein' could be seen in the guts of the insects, so there was no doubt about its having been ingested, and yet neither the ovaries nor the c.a. had increased in size; they were the same size as those of the carbohydrate controls. This suggested that the 'protein' had not been made available to the organs in question and that possibly the m.n.c. are necessary for the digestion of 'protein' materials. These results support the much stronger evidence of E. Thomsen & Møller (1959) who showed that the production of protease was dependent upon the presence of m.n.c. The fact that the c.a. remained small supports the hypothesis (E. Thomsen, 1952; M. Thomsen, 1954) that the presence of the m.n.c. is necessary for c.a. activity. In the next paper, evidence will be submitted suggesting that this influence is indirect (due to the influence of the m.n.c. upon digestion) since, in the normal fly, c.a. volume varies in proportion to the amount of 'protein' ingested.

These results and conclusions will be discussed together with additional relevant data in a later paper.

SUMMARY

1. A study has been made of the effects of removal of ovaries, corpora allata (c.a.) and median neurosecretory cells (m.n.c.) upon the selection by female blowflies of carbohydrate (sugar in water) or 'protein' (Marmite in milk).
2. Extirpation of the ovaries resulted in high carbohydrate-low protein selection and in hypertrophy of the c.a.
3. Extirpation of the c.a. resulted in low carbohydrate selection.
4. Extirpation of both ovaries and c.a. resulted in low carbohydrate selection.
5. These and other results suggest that selection is independent of direct ovarian control, but that the ovaries influence selection in that they utilize the ingested foods and thus bring about the succession of feeding cycles.
6. The c.a., whose volume (activity?) changes cyclically during each cycle of reproduction, appears to control the fluctuations in carbohydrate consumption.
7. The m.n.c. seem to be necessary for the ingestion of 'protein' and for the activity of the c.a.
8. Reproductive cycles tend to overlap. The succeeding cycle in a gravid female must be taken into consideration when events are being related to reproduction.

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