HORMONAL CONTROL OF OVARY DEVELOPMENT IN MOSQUITOES

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The control of ovary development by hormones has been shown in a number of insects, and evidence has been brought forward to demonstrate it in mosquitoes. It has been claimed on experimental grounds that a gonadotrophic hormone is secreted by the corpora allata after the intake of blood by female mosquitoes, co-ordinating ovary development with the supply of food (Detinova, 1945), and histological evidence has been produced connecting the corpora allata with ovary development (Mednikova, 1952).

Culex pipiens L. contains races which differ in their requirements for ovary development. One race is autogenous, not requiring any food during the adult stage for ovary development to occur, another race is anautogenous and the ovaries will not develop beyond a resting stage until a blood meal of adequate size has been taken. These differences must reflect differences in hormone secretion if the development of the ovaries is controlled by hormones. These and other mosquitoes have therefore been examined for evidence of humoral control of ovary development and for differences between the autogenous and anautogenous forms.

From an abundance of work it is clear that autogenous and anautogenous strains of *C. pipiens* which are genetically distinct occur in nature (Roubaud, 1930; Callot, 1947), while some strains contain both forms in varying proportions (Knight & Abdel Malek, 1951). Anautogeny seems to show incomplete dominance over autogeny (Buck, 1935; Tate & Vincent, 1936) and a unifactorial basis for the character has been questioned (Kitzmiller, 1953). The only previous explanation of the mechanism of autogeny stated that the two forms differed in the extent of the reserves carried over to the adult stage; it was considered that autogenous mosquitoes carried over sufficient reserves to develop the ovaries while anautogenous mosquitoes did not (Boissezon, 1933; Roubaud, 1933). Autogeny is known in *Drosophila*, and a similar explanation has been put forward to account for the different food requirements of *D. melanogaster* and *D. virilis* (Bodenstein, 1947).

The account which follows describes the reserves of autogenous and anautogenous mosquitoes, the evidence from histology for hormone secretion and experimental evidence for humoral control of ovary development.

MATERIAL

Culex pipiens pipiens L. Anautogenous, eurygamous (i.e. requiring a large space for copulation), bird-biting and diapausing. Cultures were maintained by collecting in Cambridge wild female mosquitoes which had already fed and mated.

Culex pipiens form *molestus* Forskål. Autogenous, stenogamous (i.e. able to copulate in a small space), man-biting and non-diapausing. This strain, which had been cultured in the laboratory for many years, was obtained from Elberfeld in Germany in 1933.

Culex pipiens form *berbericus* Roubaud. Largely anautogenous but with a very low percentage of autogeny, stenogamous, man-biting and non-diapausing. The culture was developed from eggs obtained in the Jardin d'Essai in Algiers. It was selected for anautogeny and was used as an anautogenous strain in blood-feeding experiments in which *C. pipiens pipiens* would not feed readily.

Experiments were also performed on females of *Aëdes aegypti* (L.), *Anopheles stephensi* Liston and *A. labranchiae atroparvas* van Thiel. This last species cannot be positively identified with the *A. maculipennis* sensu lato used by Detinova (1945), but it must at least be closely related to it.

THE NATURE AND EXTENT OF THE RESERVES

Although autogenous mosquitoes are considered to differ from anautogenous mosquitoes principally in the extent of the reserves carried over to the adult stage, no detailed comparison has been made of the reserves of the two forms beyond the assertion that the larvae of autogenous strains contain reserves at least three times as extensive as those of the larvae of anautogenous strains cultured under similar conditions (Roubaud & Toumanoff, 1930; Roubaud, 1933). However, poor larval nutrition has been shown to affect only the number of eggs produced by autogenous females and not the capacity to produce eggs without feeding (Gaschen, 1932; Hecht, 1933); even larvae reared at the starvation limit produced females which were all autogenous (Buck, 1935).

It is known that the fat-body cells of the larva of *Culex pipiens* contain droplets of fat, protein and glycogen (Boissezon, 1930, 1932), and a similar structure has been described in the larva of *Aëdes aegypti* (Wigglesworth, 1942). The massive abdominal muscles of the larva of *Culex pipiens* are carried over intact to the adult stage when they are autolysed, apparently providing additional protein for ovary development in the autogenous form and extending the fat-body reserves of hibernating anautogenous females (Roubaud, 1932, 1933).

Methods

Larvae of all strains were cultured in the suspensions of bacteria which develop over ground dog biscuit in water and were provided with a slight excess of food to ensure adequate nutrition. Larvae about to pupate, in which the respiratory horns had become pigmented, were chosen for examination, and the methods for staining fat-body cells described by Wigglesworth (1942) were used. To stain glycogen, specimens were fixed in Carnoy's fluid, stained in a mixture of absolute alcohol saturated with iodine (65 parts) and 1 % iodine in potassium iodide (35 parts), dehydrated in 95 % alcohol saturated with iodine and mounted in euparal. Fat and protein droplets were stained in the same specimen by fixing in Baker's formaldehyde calcium and staining first with Sudan IV and then with ninhydrin.

The reserves of autogenous and anautogenous Culex pipiens

Dissection of fully grown fourth-instar larvae showed that the fat-body lobes of *Culex pipiens pipiens* were rather less extensive than those of *C. pipiens* form *molestus*, but that they were developed to a sufficient extent to contain a considerable amount of reserves. The abdominal muscles were extensively developed in both forms. No differences were found in the nature or extent of the reserves within the fat-body cells. Before pupation the cells were packed with large droplets of fat and smaller droplets of protein and glycogen. The difference in the extent of the fat body appeared slightly greater in the adult, but it was clear that the anautogenous *C. pipiens pipiens* contained sufficient reserves to develop a number of eggs, for the fat body contained considerable quantities of protein and the abdominal muscles provided much more.

This study of the fat body led to interesting observations on its coloration. In well-nourished cultures the fat body of the fully grown larva was often strongly pigmented, green in *C. pipiens* form *molestus* and pink or purple in *C. pipiens* form *berbericus*. The coloration was found to be confined to the protein droplets, and when Gmelin's reagent was added to exposed fat-body cells the pink colour turned to green and then to blue, while the green-coloured fat body developed a bluish colour. This suggested that the colours were due to bile pigments. The fat body was pigmented in the adult mosquitoes at emergence, but within a day the pigment disappeared from the fat body and accumulated in the pericardial cells. Strains of *C. pipiens* have been described with the larval fat body coloured green and brown, the colour forms being inherited in simple Mendelian ratios when the strains were crossed (Huff, 1929).

The mobilization of the reserves

The fate of the reserves contained in the fat body and the abdominal muscles during the first 5 days of adult life is shown in Table 1, together with the state of the ovaries. The extent of the fat body is expressed by the numbers 5 (extensive) to 1 (very slight), that of the abdominal muscles by the numbers 4 to 0. The categories of ovary development described by Christophers, Sinton & Covell (1936) are used to show the state of the ovaries. The ovaries of anautogenous mosquitoes enter a resting stage during stage II of Christophers *et al.*, and further growth is dependent on blood feeding. For convenience, stage II has been divided into stage II*a* (ovaries entering or in the resting stage) and stage II*b* (ovaries which have passed the resting stage but which have not reached stage III).

The ovaries of *C. pipiens* form *molestus* were seen to develop to maturity within 5 days; concurrently the fat body became greatly reduced and the abdominal muscles were autolysed, disappearing completely by the second day after emergence (Table 1). In *C. pipiens pipiens* the ovary did not develop beyond the resting stage in the absence of a blood meal, but both fat-body and muscle reserves were mobilized. The apparent increase of the fat body after emergence was probably due to the variability of the material. It may be noted that decapitation and ligation of the abdomen of *C. pipiens* form *molestus* within an hour of emergence did not prevent the utilization of the fat-body reserves or the breakdown of the abdominal muscles.

Table 1. *The state of the ovaries, fat body and abdominal muscles of females of autogenous and anautogenous forms of* Culex pipiens *under various conditions. Each daily entry is the record of five individuals and shows the range of observations on ovary development and the mean of the observations on fat body and muscle*

Species	Organ	Days after emergence					
		0	1	2	3	4	5
C. pipiens form *molestus* unfed	Ovary	I	II*a*	II*b*–III	III	IV	V
	Fat body	5	4·8	4·2	2·1	1	1
	Muscles	4	2·8	0	0	0	0
C. pipiens pipiens unfed	Ovary	I	I	I	I–II*a*	I	I–II*a*
	Fat body	2·8	4	3·6	1·8	1·4	1
	Muscles	4	2	0	0	0	0
C. pipiens form *molestus* decapitated within 1 hr. of emergence	Ovary	—	I	I–II*a*	I–II*a*	I–II*a*	I–II*a*
	Fat body	—	4·2	3·4	2·2	2·2	1·8
	Muscles	—	2·4	0·2	0	0	0
C. pipiens form *molestus* abdomen ligated within 1 hr. of emergence	Ovary	—	I	I	I–(II*b*)*	I	I–II*a*
	Fat body	—	3·6	3·4	3·2	2·6	2·2
	Muscles	—	1·2	0·8	0	0	0
Species	**Organ**	**Days after blood meal**					
		0	1	2	3	4	5
C. pipiens form *berbericus* fed human blood	Ovary	—	II*b*–III	III	III–V	III–V	V
	Fat body	—	1·8	1·8	1·6	1	1
	Muscles	—	0	0	0	0	0

* One specimen had two follicles at stage II*b* and its remaining follicles at stages I or II*a*.

Staining the inclusions of the fat-body cells showed that the various reserves were not all used concurrently. The 48-hour-old female of *C. pipiens* form *molestus* had abundant fat, glycogen and protein in its fat-body cells, at 72 hr. there was very little protein but much fat and glycogen, and at 120 hr., when the eggs were mature, there was no protein and only traces of fat and glycogen. Females of this age, in which ovary development had been prevented by ligation, contained some fat and glycogen and abundant protein. The fat-body cells of females of *C. pipiens pipiens* 96 hr. after emergence contained small amounts of fat and glycogen and appreciable amounts of protein. After 120 hr. there were only traces of fat and glycogen and no protein.

THE HISTOLOGY OF THE ENDOCRINE ORGANS

Little is known of the structure and function of the endocrine organs of mosquitoes. Their structure has been described in part (Bodenstein, 1945; Cazal, 1948) and the corpora allata have been linked with ovary development on histological grounds. Detinova (1945) found that the size of the corpora allata of *Anopheles maculipennis* sensu lato rapidly decreased after the intake of blood, and Mednikova (1952) deduced from a histological study that the corpora allata stimulated ovary development in *Anopheles* and *Chaoborus*. In the present work, female pupae and adults of *Culex pipiens* were fixed in aqueous Bouin and stained with Gomori's chrome haematoxylin. Other stains used were Masson's trichrome stain and Samuel's silver stain.

Neurosecretory cells

Neurosecretory cells were found in the supra- and suboesophageal ganglia and in the thoracic and abdominal ganglia in pupae and adults of *Culex pipiens*. In *Calliphora*, the neurosecretory cells of the supraoesophageal ganglion have been shown to play an important part in ovary development (Thomsen, 1952), and particular attention was paid to these cells in *Culex pipiens*. A group of about ten neurosecretory cells occurred in the pars intercerebralis, and others were to be found in different parts of the supraoesophageal ganglion. The cytoplasm of the cells contained numerous granules which stained blue with Gomori's chrome haematoxylin, but the axons of the cells could be traced only a short distance and no neurosecretory granules could be seen in them. The neurosecretory cells were closely examined for signs of secretion in *C. pipiens* form *molestus* during the 24 hr. after emergence, in which period experiments showed ovary development was stimulated, and in *C. pipiens* form *berbericus* during the 24 hr. following blood feeding. However, no changes in the granular content of the cytoplasm could be seen.

Peritracheal glands and corpora allata

The peritracheal glands of *C. pipiens*, which surround the corpora allata, degenerate during the late pupal stage and are unlikely to play any part in ovary development.

The corpora allata were closely examined for secretory activity in females of *C. pipiens* form *molestus* during the 24 hr. after emergence and in *C. pipiens* form *berbericus* during the 24 hr. after feeding on blood. No change in the volume of the corpora allata was apparent over these periods. In certain individuals, the corpora allata had a vacuolate appearance and the vacuoles occasionally contained chromatic droplets stained black by Gomori's chrome haematoxylin (Fig. 1 *a*), but the random occurrence of this condition suggested that it was not connected with the development of the ovaries.

Corpora cardiaca

Cazal (1948) described an unpaired corpus cardiacum, in the pupa of *C. pipiens*, fused with the hypocerebral ganglion and lying below the aorta in the neck. Examination of the hypocerebral ganglion, in pupae stained with Gomori's chrome haematoxylin after fixation in aqueous and alcoholic Bouin, showed a group of cells, similar to brain cells, lying below the aorta in the neck region and receiving the recurrent nerve and the two cardiacal nerves from the brain. Cells were found, close to the hypocerebral ganglion, which resembled those figured by Cazal as part of the corpus cardiacum. These cells could be seen surrounding the pharynx in this region, apparently forming part of the wall of the pharynx. In the pupa there is a group of these cells just below the hypocerebral ganglion, but in the adult, not examined by Cazal, they become flattened into a single layer.

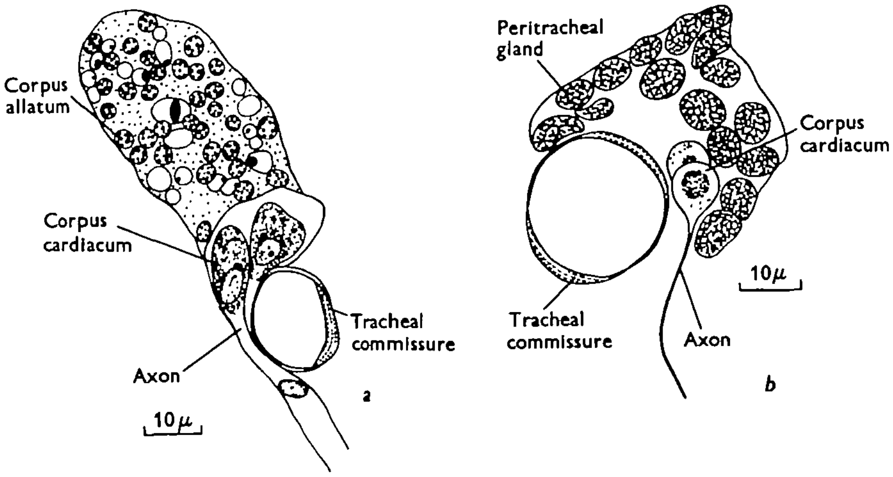

Fig. 1 *a*, *b*. Endocrine organs of *Culex pipiens*. *a*. Sagittal section of the corpus allatum and corpus cardiacum of an adult female of *C. pipiens* form *berbericus*, stained with Gomori's chrome haematoxylin. *b*. Sagittal section of the peritracheal gland and corpus cardiacum of a female pupa of *C. pipiens* form *molestus*, stained with Samuel's silver stain.

Two groups of cells, previously undescribed, are to be found between the corpora allata and the tracheal commissure (Fig. 1 *a*). Each group consists of three or four large cells with large nuclei and extensive cytoplasm containing granular material which stains with Gomori's chrome haematoxylin. From these cells axons run forward under the tracheal commissure in the cardiacal nerve. The axons, consistently found in Gomori-stained sections, were also found in pupal material stained with Samuel's silver stain after fixation in the recommended modification of Bouin (Samuel, 1953) (Fig. 1 *b*). In the late fourth-instar larva, some cells with extensive, clear cytoplasm, which probably corresponded with the newly described cells found in the pupa and adult, were occasionally seen between the peritracheal gland cells near the tracheal commissure. The position of these two groups of cells and their paired condition suggest that they comprise the corpora cardiaca.* Axon-like structures, running from the cells of the corpora cardiaca into the cardiacal nerves, have been described in the larva of *Ptychoptera* (Thomsen, 1951).

No evidence was obtained to suggest that the corpora cardiaca were active in ovary development.

* That the bodies are cells and not swollen nerve endings has been confirmed by phase contrast examination of living material. The 'nerve' appears to be a homogeneous protoplasmic trunk surrounded by a very fine sheath.

EXPERIMENTS ON OVARY DEVELOPMENT

Detinova (1945) claimed that ligation of *Anopheles maculipennis* sensu lato after a blood meal did not prevent ovary development if the ligature was tied between the head and thorax, but did prevent ovary development if it was tied between the pro- and mesothorax within 6 hr. of feeding. From this result she concluded that the corpora allata stimulated ovary growth by secreting a gonadotrophic hormone after blood feeding. Similar experiments have been performed on the autogenous mosquito, *Culex pipiens* form *molestus*, after emergence of the adults, and on the following anautogenous mosquitoes after blood-feeding, *C. pipiens* form *berbericus*, *Aëdes aegypti*, *Anopheles labranchiae atroparvus* and *A. stephensi*. The strain of *Culex pipiens* form *berbericus*, which was largely anautogenous and selected for anautogeny, was used instead of the entirely anautogenous *C. pipiens pipiens* because the latter is very difficult to feed under experimental conditions. Mosquitoes were decapitated or were ligated at the base of the abdomen using a fine hair. Decapitation removes the neurosecretory cells of the brain and severs the cardiacal and recurrent nerves, while ligation isolates the ovaries from all the endocrine organs outside the abdomen. In the later ligation experiments, those performed on *Anopheles stephensi*, *A. labranchiae atroparvus* and *Aëdes aegypti*, the head and the front of the thorax were cut away after the ligature had been tied, but no change in result was observed. The ovaries of the decapitated mosquitoes were examined 5 days after operation, and those of ligated specimens, which survived less well, after 3 days.

Results

Females of *Culex pipiens* form *molestus* were decapitated or ligated at the base of the abdomen at hourly intervals after emergence from the pupa, and the subsequent ovary development of these mosquitoes was studied (Figs. 2, 3). No specimens decapitated earlier than 7 hr. after emergence developed their ovaries beyond the resting stage, while many of those decapitated more than 7 hr. after emergence developed their eggs to maturity or to a lesser degree. Among the ligated specimens, none ligated earlier than 5 hr. after emergence developed its ovaries beyond the resting stage, but many of those operated on more than 5 hr. after emergence did so.

When the anautogenous females of *C. pipiens* form *berbericus* were decapitated immediately after feeding on human blood, a proportion of them developed their ovaries beyond the resting stage. This was in accordance with the result obtained by Detinova (1945) in similar experiments on *Anopheles maculipennis* sensu lato. The result obtained by ligating the abdomens of females of *Culex pipiens* form *berbericus* after blood feeding was quite different from that obtained by Detinova. Females ligated as soon as 3–4 min. after the *start* of blood feeding developed their ovaries beyond the resting stage (Fig. 4).

Ligation experiments were also performed on other anautogenous species of mosquito after feeding on human blood. Females of *Aëdes aegypti* which had been ligated as early as 2–3 min. after the start of feeding developed their ovaries beyond the resting stage (Fig. 5), and females of *Anopheles labranchiae atroparvus* ligated 1–2 min. after starting to feed developed their ovaries beyond the resting stage (Fig. 6).

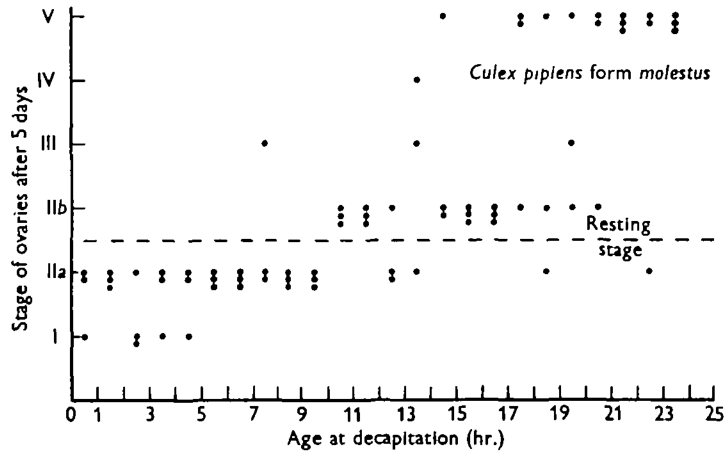

Fig. 2. The effect of decapitation on the development of the ovaries of *Culex pipiens* form *molestus* when performed at hourly intervals after emergence. Each point represents one female.

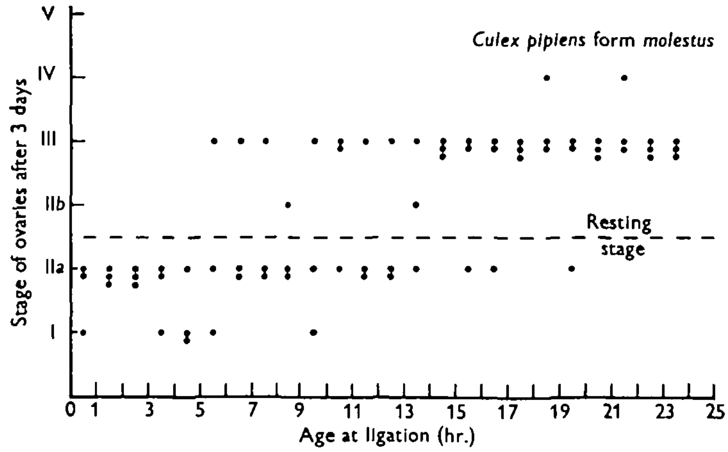

Fig. 3. The effect of ligation on the development of the ovaries of *Culex pipiens* form *molestus* when performed at hourly intervals after emergence. Each point represents one female.

A. stephensi was the only anautogenous mosquito which gave results similar to those described by Detinova for *A. maculipennis* sensu lato. Fifty specimens (of which only a few are shown in Fig. 7) ligated within 1 hr. of feeding did not develop their ovaries beyond the resting stage. Among those ligated 2 or more hours after feeding occasional specimens developed their ovaries to stage II *b*, and among females ligated 9 hr. after feeding a considerable proportion reached this stage. From 18 hr. onwards a number of specimens developed their eggs to maturity.

Implantation experiments

The anautogenous females of *Culex pipiens pipiens* had been shown to contain sufficient reserves for the development of a number of eggs without the addition of protein from ingested blood, and an attempt was made to stimulate the development

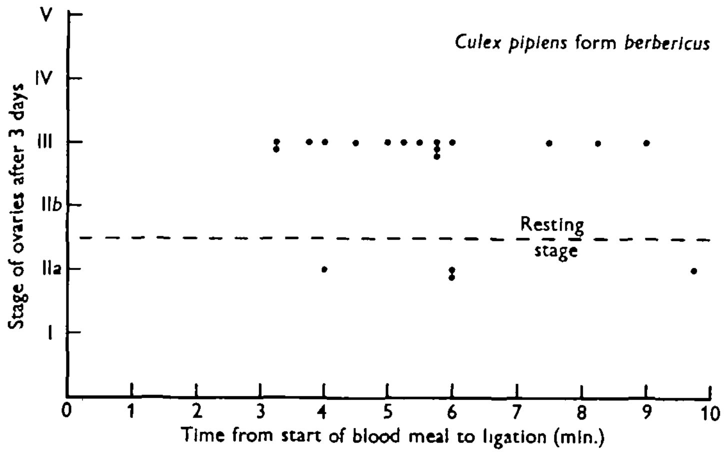

Fig. 4. The effect of ligation on the development of the ovaries of *Culex pipiens* form *berbericus* when performed immediately after blood feeding. Each point represents one female.

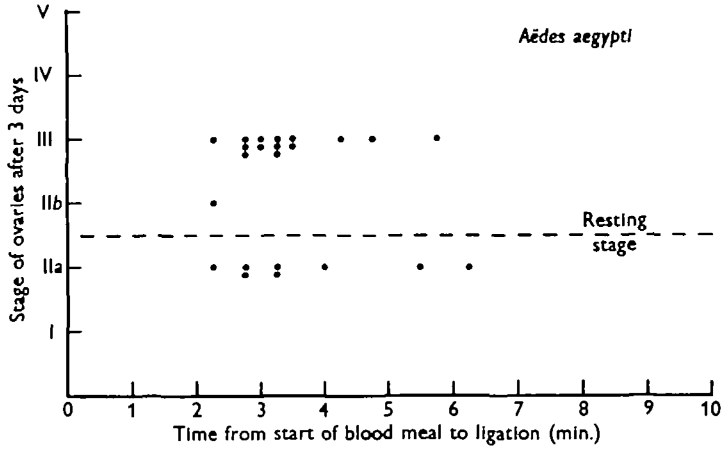

Fig. 5. The effect of ligation on the development of the ovaries of *Aëdes aegypti* when performed immediately after blood feeding. Each point represents one female.

of the ovaries of unfed females by implanting active endocrine organs. In early experiments brains, corpora allata and corpora cardiaca, removed from females of *C. pipiens* form *molestus* a few hours after emergence, were transplanted into the abdomens of females of *C. pipiens pipiens*. No positive results of ovary development were obtained, but the difficulty of handling the small endocrine organs suggested that the transplantations were not successfully performed.

Thomsen (1952) prevented ovary development in *Calliphora erythrocephala* by removing the median neurosecretory cells from young females, and was able to restore ovary development in a small proportion of the operated flies by transplanting, into each, three corpora allata-cardiaca systems from female flies fed meat for

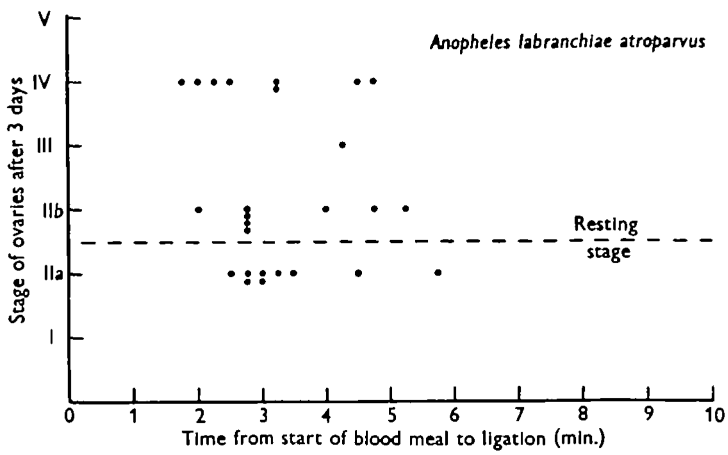

Fig. 6. The effect of ligation on the development of the ovaries of *Anopheles labranchiae atroparvus* when performed immediately after blood feeding. Each point represents one female.

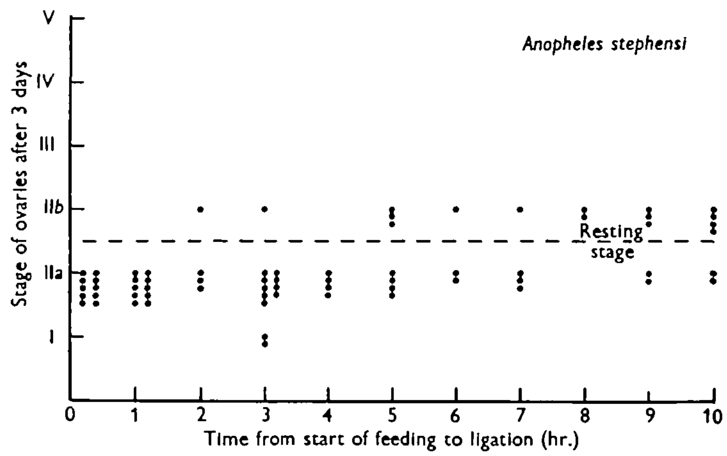

Fig. 7. The effect of ligation on the development of the ovaries of *Anopheles stephensi* when performed at various times after blood feeding. Each point represents one female.

7 days. Similar transplantations were made into sugar-fed females of *Culex pipiens pipiens*, three corpora allata-cardiaca systems from females of *Calliphora* fed meat for 6–7 days being implanted into the abdomen of each mosquito. However, in twenty-seven individuals into which the endocrine organs had been successfully implanted and which lived for 5 days after the operation, no development of the ovaries beyond the resting stage was found.

DISCUSSION

Autogeny in *Culex pipiens* form *molestus* has been said to result from the accumulation of additional reserves during the larval stage. As the anautogenous form, *C. pipiens pipiens*, has now been shown to contain considerable reserves, some other explanation of autogeny must be looked for. Both decapitation of *C. pipiens* form *molestus* and ligation at the base of the abdomen, when performed within a few hours of emergence, prevented the development of the ovaries which would normally have taken place. The simplest explanation of this result is that the operation prevents the working of a gonadotrophic hormone. This explanation is not supported by additional evidence from histology or from implantation experiments, but it is not inconsistent with what is already known of insect hormones. Decapitation removes the neurosecretory cells and cuts the cardiacal nerves and the recurrent nerve, while ligation isolates the ovaries from all endocrine organs outside the abdomen. There is thus sufficient experimental evidence to suggest that the development of the ovaries in autogenous mosquitoes is stimulated by a gonadotrophic hormone secreted within a few hours of emergence, presumably by the corpora allata.

Detinova (1945) claimed that in *Anopheles maculipennis* sensu lato ovary development was stimulated by the secretion of a gonadotrophic hormone by the corpora allata some hours after feeding on blood, and that ovary development could be prevented by ligating behind the corpora allata. In three species of mosquito ligation failed to prevent ovary development when it was performed immediately after feeding, in some cases 2–3 min. after the start of feeding. Only in *A. stephensi* did there appear to be a critical period, the ovaries developing only when the ligature was tied 2 hr. or more after feeding. If the critical period in *A. stephensi* represents a period of hormone secretion, then the situation appears only slightly different from that in *Culex pipiens* form *molestus*, the blood meal stimulating secretion of the gonadotrophic hormone instead of secretion occurring automatically after emergence.

On the present evidence, no straightforward theory of hormone secretion can be applied to the other species, *Culex pipiens* form *berbericus*, *Aëdes aegypti* and *Anopheles labranchiae atroparvus*. It seems unlikely that, where ovary development takes place despite ligation within a very few minutes of the start of feeding, sufficient hormone can pass into the abdomen. If the hormones are secreted before feeding takes place, some chemical factor in the food or some behavioural stimulus from feeding may trigger ovary growth. A chemical factor in the blood has been sought by a number of workers, but there is little evidence to suggest that more than protein is required (Yoeli & Mer, 1938; Greenberg, 1951; Hosoi, 1954). Clearly, further evidence is necessary for an understanding of the control of ovary development in these anautogenous mosquitoes.

SUMMARY

A study was made of ovary development in *Culex pipiens* form *molestus* Forskål, an autogenous mosquito not needing food in the adult stage to develop its eggs, and in *C. pipiens pipiens* L. and other anautogenous mosquitoes which require blood for ovary development.

Comparison of the reserves of fully grown larvae of the autogenous and anautogenous forms of *C. pipiens* showed that the autogenous form had a rather larger fat body, but that the anautogenous form contained sufficient protein reserves to develop a number of eggs. It was considered that autogeny did not depend solely upon the ability to amass extensive reserves but also upon some other physiological mechanism.

Decapitation and ligation at the base of the abdomen prevented ovary development in *C. pipiens* form *molestus* when performed within a few hours of emergence, but when performed 7 or more hours after emergence it often failed to prevent ovary development. It is suggested that a gonadotrophic hormone is secreted during this time.

Ligation of the abdomen within an hour of feeding on blood appeared to prevent ovary development in *Anopheles stephensi* Liston. Ovary development occurred in a small proportion of females ligated 2 or more hours after feeding, and this proportion increased with time.

Ligation of the abdomen immediately after blood feeding failed to prevent ovary development in *Culex pipiens* form *berbericus* Roubaud, *Aëdes aegypti* (L.) and *Anopheles labranchiae atroparvus* van Thiel, even in some cases where the ligature was tied within 2–3 min. of the start of feeding.

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