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THE CORPUS ALLATUM AND OOGENESIS IN *RHODNIUS PROLIXUS* (STÅL.)

III. THE EFFECT OF MATING

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INTRODUCTION

A virgin female *Rhodnius* produces only about two-thirds the number of eggs produced by a mated female (Buxton, 1930; Coles, 1965; Davey, 1965). Some, at least, of this effect is upon oviposition, which proceeds more slowly in the virgin female. Earlier studies from this laboratory (Davey, 1965, 1967, 1970) have suggested that this response to mating does not depend on the presence of the corpus allatum, but is mediated by a blood-borne substance released from mated spermathecae which in turn brings about the release of a myotropin from the neurosecretory cells of the pars intercerebralis of the brain.

Quite apart from these effects on oviposition it is important to realize that mated females *produce* more eggs than do virgin females (Davey, 1965). It therefore seemed possible that two separate endocrine factors operate on oogenesis in the mated female, and that one of these (not mediated by the corpus allatum) is lacking in the virgin. We have therefore examined the time-course of changes in size of oocytes in the ovarioles from virgin females and compared this with similar measurements performed on mated and on mated-allatectomized females.

In the first paper in this series (Pratt & Davey, 1972*a*) we have identified certain stages within the ovarioles as being sensitive to allatectomy. Oocytes in allatectomized ovarioles pass through the critical size range just below 400 μm less readily than in normal ovarioles; there is thus a 'reluctance' to enter vitellogenesis. Once the oocytes have grown beyond 400 μm (at which point the follicle layer becomes patent and vitellogenesis begins) they grow more slowly; there is thus a reduced rate of vitellogenesis.

MATERIALS AND METHODS

Details of the procedures used to measure the oocytes will be found in an earlier paper (Pratt & Davey, 1972*a*). In general, we have measured the length of the three most terminal oocytes (the terminal, or T, oocyte, the penultimate, or T-1, and the antepenultimate, or T-2) in each ovariole for not fewer than five animals at each day investigated. We have grouped the oocytes into four classes: (1) those less than 300 μm long; (2) those 300–400 μm long, which is the stage just prior to vitellogenesis and

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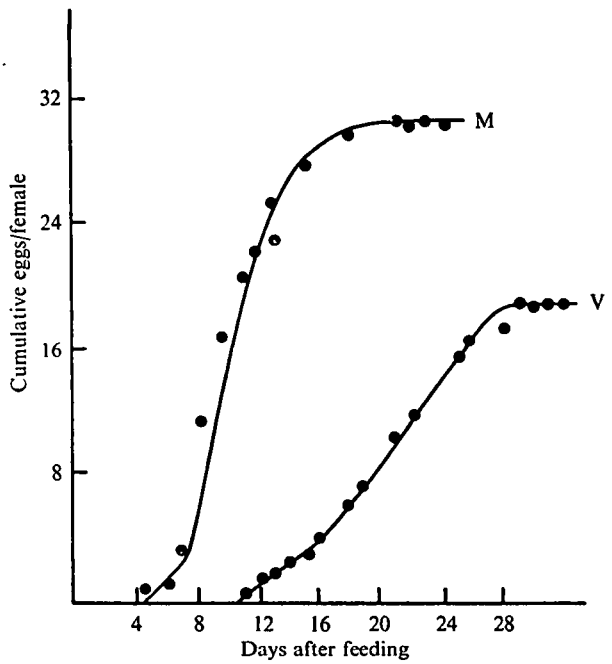
which is sensitive to the presence of the allatum hormone; (3) those greater than 400 μm in length, but without a chorion – the vitellogenic stage; (4) those in which the deposition of the chorion has begun.

Farnesyl methyl ether (FME) was applied topically according to the method of Wigglesworth (1969). The sample used in our experiments was the all-trans isomer and was assayed by the technique of Wigglesworth (1969). The juvenilizing potency was identical to that determined by Wigglesworth (1969) for a similar sample.

RESULTS

Previous results from this laboratory have been based upon the average egg production per female over at least four cycles of egg production in females fed every 10 days (Davey, 1965, 1967). Text-fig. 1 shows a comparison of the rate of oviposition

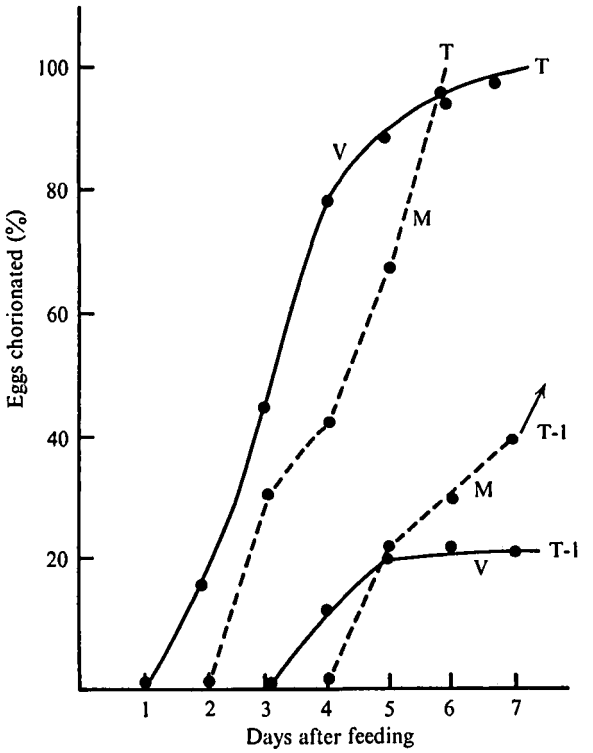


Text-fig. 1. Cumulative numbers of eggs laid per virgin (V) or mated (M) female during the cycle of oviposition following the first meal.

of mated and virgin females on feeding regimes in which all of the eggs produced are laid before the second meal. Note not only that the number of eggs laid by a mated female is larger, but also that oviposition begins earlier and proceeds more rapidly than in a virgin female. A virgin female produces on the average some 17 or 18 eggs, or slightly more than one for each of the 14 ovarioles. By contrast the mated females produce about 30 eggs, or more than two per ovariole.

The effect of mating upon successive waves of oocytes

Text-fig. 2 shows the percentage of ovarioles in which the T or T-1 oocyte has deposited a chorion on each of the first 7 days after feeding. This represents an approximate index of the rate of growth of the two waves of vitellogenesis in each ovariole.

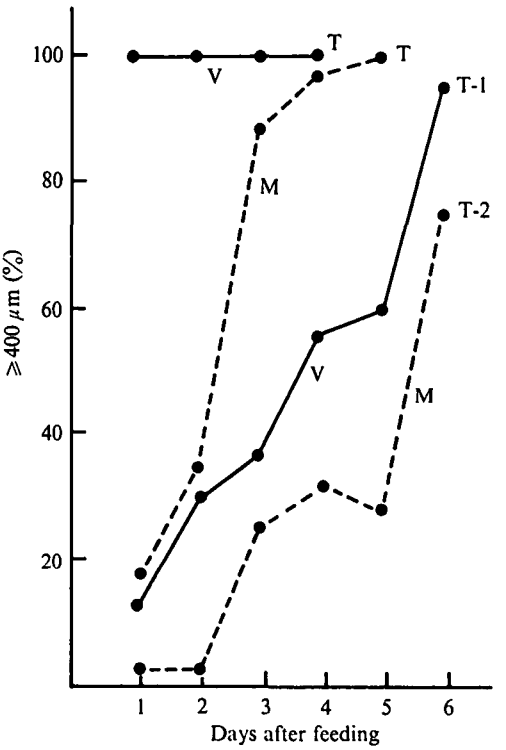


Text-fig. 2. The percentage of T or T-1 oocytes which have been chorionated in ovarioles taken from mated (dotted lines, M) or virgin (solid lines, V) females at various times after feeding. The points represent the means of determination for at least five animals.

From this graph it is clear that the rate of growth of the T oocytes in virgins is at least equal to or greater than the rate of growth of the T oocytes in mated females. On the other hand, the T-1 oocytes of the virgins are almost completely inhibited. It is not possible to carry the observations on mated females beyond the seventh day, as oviposition intervenes. Thus, of the oocytes the first wave reach maturity in both virgin and mated females at about the same rate. It is only in the growth of the oocytes of the second wave that differences appear. The lower rate of egg production in a virgin female appears to stem exclusively from an almost total inhibition of the growth of the T-1 oocytes.

Text-fig. 3 examines the percentage of ovarioles which contain T or T-1 oocytes which are greater than 400 μm and have therefore passed through the stage of activation which we have already shown to be a prerequisite for vitellogenesis (Pratt & Davey, 1972*a*). All of the T oocytes of virgins are beyond the stage of incipient

activation within 1 day of feeding, whereas very few of the T oocytes have achieved this size in mated females. This situation clearly arises from conditions prior to the period of observation and the meal, and is unrelated to the fact that these virgins will go on to produce fewer eggs. The explanation for this phenomenon probably lies in a slower rate of digestion in virgins of the blood remaining from the pre-moult meal and the consequent relatively less starved condition of virgin females.



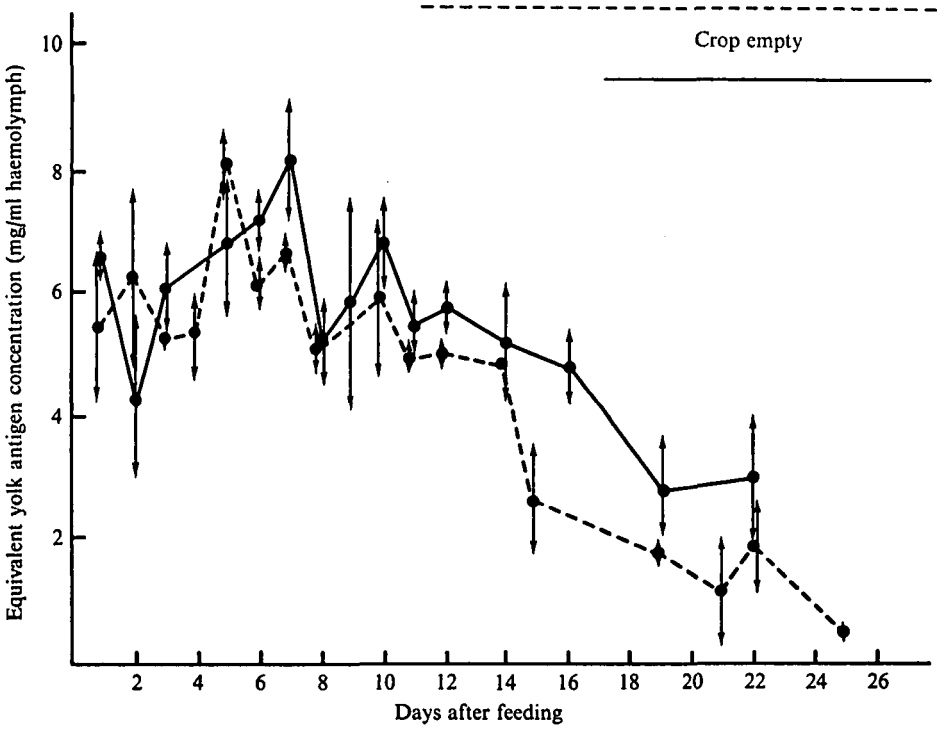
Text-fig. 3. The percentage of ovarioles from mated (M, dotted lines) or virgin (V, solid lines) which contain T or T-1 oocytes larger than 400 μm. Each point represents the mean for at least five animals.

We have measured these virgin T-1 oocytes up until the ninth day after feeding. By this time they will have undergone vitellogenesis to varying degrees and may have attained a length of up to 800 μm. However, apart from three or four which were activated at a favourably early time and were completed by day 5 or 6, none of these T-1 oocytes will complete vitellogenesis. Either starvation-induced resorption or a second meal will intervene.

The effect of mating upon the yolk protein of the haemolymph

An earlier paper (Pratt & Davey, 1972*a*) demonstrated a reduced rate of vitellogenesis in surgically allatectomized animals, and associated this reduction with a reduced titre of yolk protein in the haemolymph. Text-fig. 4 plots the concentration of yolk protein in the haemolymph of mated and virgin females. There is no evidence of a

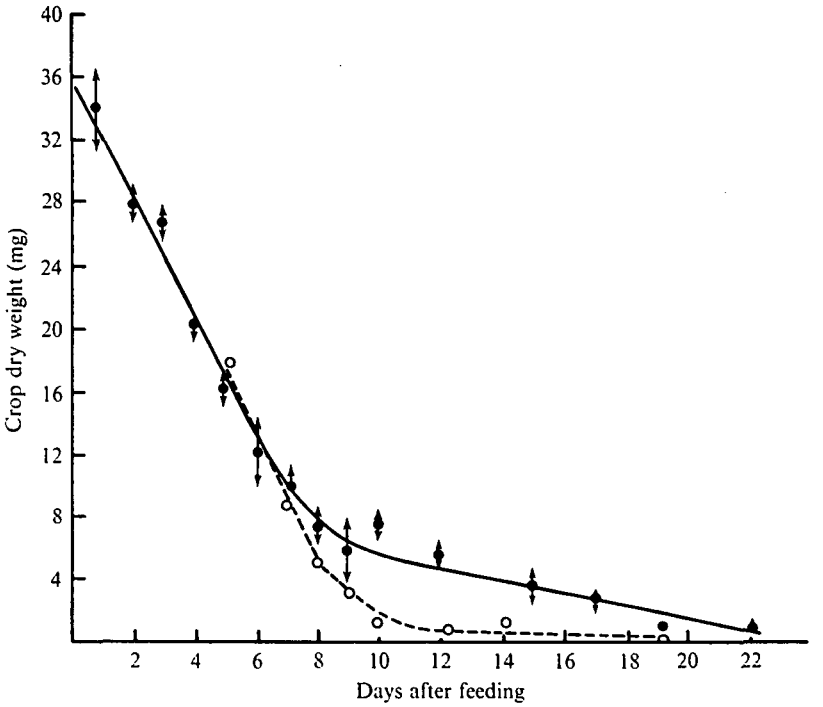
precocious decline in virgins associated with their early termination of egg production. Indeed, the reverse situation may obtain; the concentration remains at a higher level for longer in virgins. The ultimate decline, which in virgins occurs 10 days after the arrest of vitellogenesis, may be more usefully correlated with the onset of starvation, and the time at which the crop becomes empty is therefore included in Text-fig. 4. Thus, in the unoperated female, it is clear that the regulation of the rate of vitellogenesis and of the concentration of the yolk protein are separable phenomena.



Text-fig. 4. The equivalent concentration of yolk protein in the haemolymph of mated (dotted line) and virgin (solid line) females at various times after feeding. The lines have been drawn through the means of determinations and the arrows indicate the standard errors. The horizontal lines at the top of the graph indicate the times in the cycle when the crop becomes empty.

Crop emptying

We have repeated and extended Coles's (1965) observations on the decline of crop dry weight in mated and virgin females. Text-fig. 5 confirms that up to the seventh day after the meal the rates of crop emptying are identical in mated and virgin females. However, it is at about this time that vitellogenesis ceases in virgin females. In extending the period of observation we have found that whereas in mated females crop emptying proceeds to completion, in virgins there is a dramatic reduction in rate, beginning at day 7 or 8, so that the remaining crop contents (22% of the blood meal) are released much more slowly. The crop finally becomes empty at about 20 days after the meal in virgins, whereas in mated females it is empty by the twelfth day after the meal. Thus virgin females enter starvation very much later than do mated females.



Text-fig. 5. The decline in crop dry weight with time after feeding in mated (dotted line, open circles) and virgin (solid lines, solid circles) females. The points indicate the means, and where the standard errors lie beyond the area covered by the points these are indicated by arrows. Only females which imbibed meals weighing 180–220 mg are included in these data.

Patency in the follicular epithelium

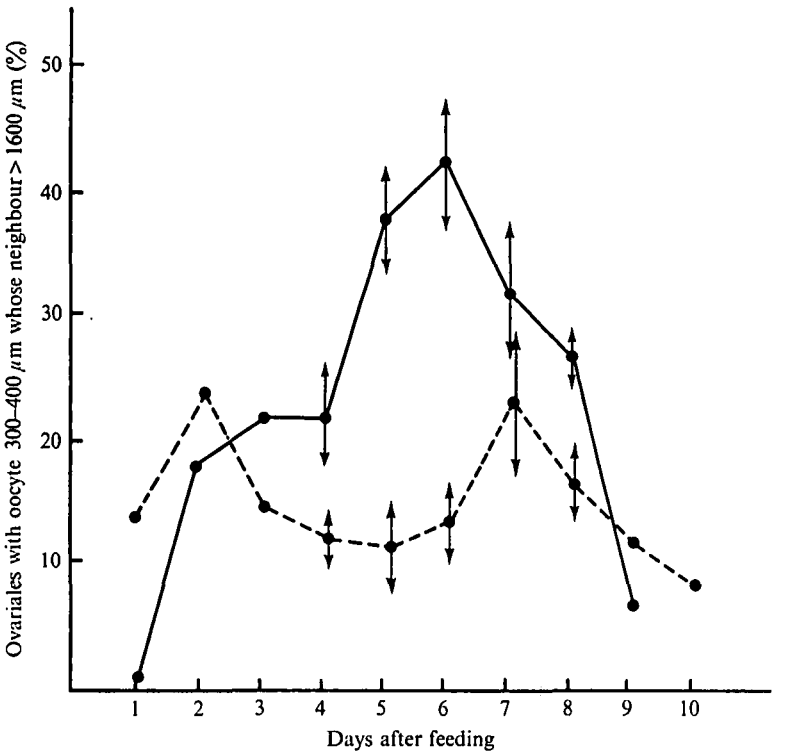
An earlier study (Patchin & Davey, 1968) reported that when vitellogenesis wanes in the normal animal there is an obvious decrease in the quantity of protein located in the intercellular spaces of the follicular epithelium of those follicles which are undergoing vitellogenesis. Using the penetration of Evans blue into the interfollicular spaces as a criterion of patency (Pratt & Davey, 1972*a*) it is clear that a large follicle of the first wave of oocytes in a virgin female has spaces between virtually all the follicle cells (Pl. 1, fig. 1). In a T-1 oocyte of similar size, however, tripartite junctional spaces are more sparsely scattered over the surface of the follicle (Plate 1, fig. 2). The loss of patency must be an important component of the inhibition operative in the T-1 follicles of virgins.

The accumulation of pre-vitellogenic oocytes in the virgin

It is clear from the foregoing that the T-1 oocyte in a virgin *enters* vitellogenesis as rapidly as that in a mated female. Once embarked upon vitellogenesis, however, the T-1 oocytes grow more slowly in the virgin, possibly as a result of the more sparsely scattered intercellular spaces in the follicular epithelium. In this respect the T-1 oocytes behave like the oocytes in an allatectomized animal (Pratt & Davey, 1972*a*).

In the allatectomized animal there is a delay in the activation of oocytes, resulting in a transient accumulation in the 300–400 μm size range (Pratt & Davey, 1972*a*). It is

already clear that there is no delay in the activation of the T-1 oocyte, but what of its younger neighbour, the T-2 oocyte? Because of the internal co-ordination of growth within the ovariole (Pratt & Davey, 1971*a*) there will be no oocyte which grows beyond 400 μm in length unless its elder neighbour has already exceeded a length of 1600 μm (Pratt & Davey, 1972*a*). Thus, in Text-fig. 6 only those T-2 oocytes have been included whose elder neighbours have already exceeded 1600 μm in length. This is a very large correction since we have already shown that, in a virgin, there is a tendency to impose stasis upon the T-1 oocyte. In spite of the application of this correction, there is evidence of a transient synchrony of T-2 oocytes in the pre-activation size range between days 5 and 8.



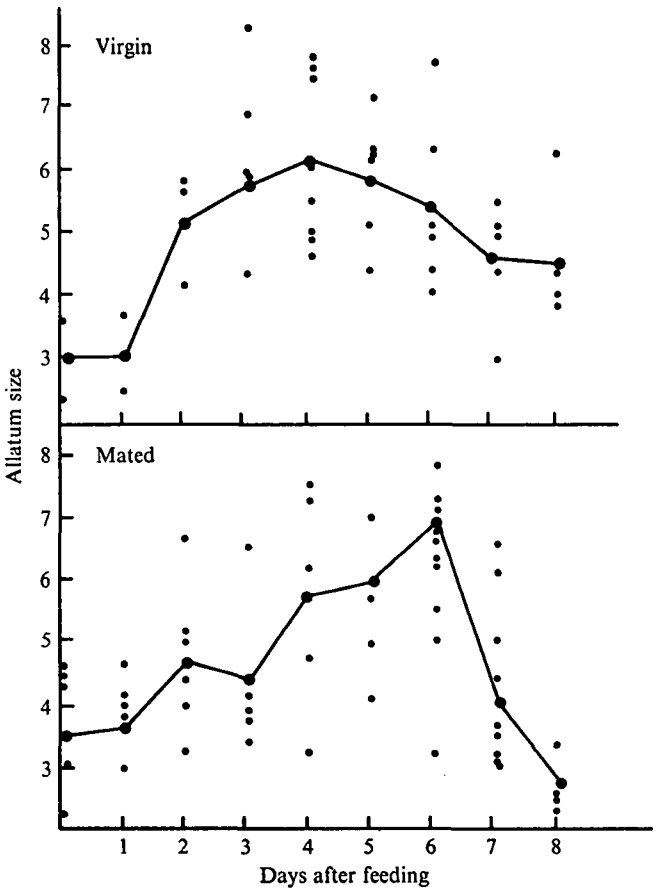
Text-fig. 6. The percentage of ovarioles with an oocyte 300–400 μm long in virgin (solid line) and mated (dotted line) females at various times after feeding. Only those oocytes whose eldest neighbour is greater than 1600 μm are included. Arrows show 90% fiducial limits.

Thus, during the time when vitellogenesis is inhibited in virgins, there is also an inhibition of the rate of transition of younger oocytes. In both of these respects the virgin female resembles the allatectomized female. Previous work (Davey, 1967), however, has suggested that the allatum is not involved in the stimulation of egg production attendant upon mating.

The size of the corpus allatum in mated and virgin females

The size of the corpus allatum has been used by many workers as an index of the activity of that organ. We have measured the major and minor diameters of the gland

in virgin and mated females up to the ninth day after feeding. The corpora allata were dissected, along with the cardiaca and part of the heart, from preparations fixed in 4% glutaraldehyde and mounted in a commercial mounting medium (CMCS 10: Turtox, Chicago, Ill., U.S.A.). Text-fig. 7 shows that there is a cyclical increase in size, expressed as arbitrary units, but there is no difference between mated and virgin females which we can relate convincingly to the different activities of their respective ovaries.



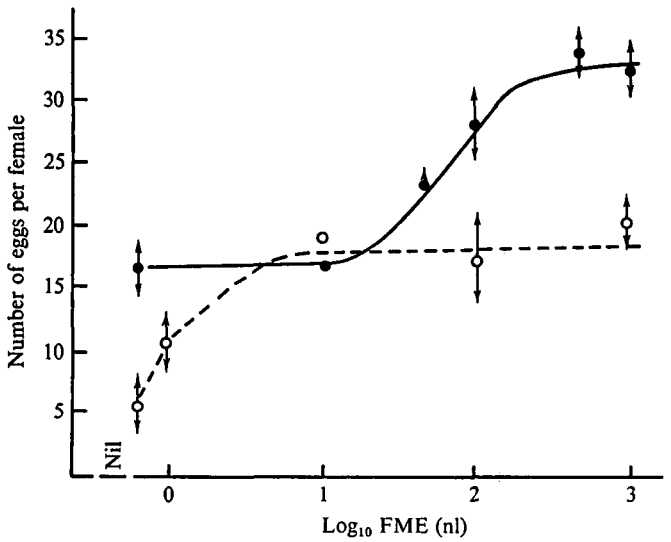
Text-fig. 7. The size of corpora allata, expressed as arbitrary units, in mated (lower curve) or virgin (upper curve) females at various times after feeding. The individual measurements have been plotted, and the lines are drawn through the means for each day.

The effect of FME on virgin ovaries

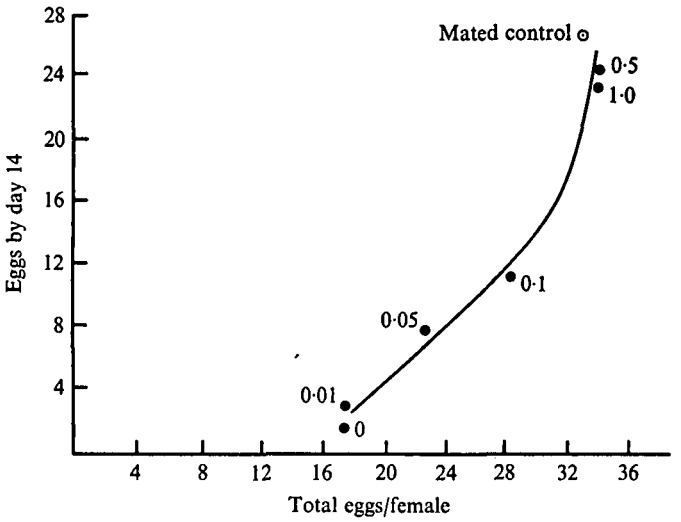
Another experimental approach to the hypothesis that a virgin female is in some way deficient in the gonadotropin from the corpus allatum involves the use of farnesyl methyl ether, similar in action to juvenile hormone, as a replacement for the supposed deficiency. Two experiments were performed; in one the FME was applied on the fourth day after the meal and in the other on the eighth day. The total egg production of the females was then determined. The responses were identical and the results have been pooled and presented in Text-fig. 8 along with the results of a similar experi-

ment in which FME was applied to mated females which had been decapitated immediately after feeding. A range of doses was employed.

From these results it is clear that a mated, decapitated female under the influence of FME produces only about the same number of eggs as is characteristic of a normal virgin. It is possible to increase the rate of egg production of virgin females by treatment with FME, but the dose required is at least 50 times that required to elicit the maximum possible response in a decapitated female, and more than 100 times the



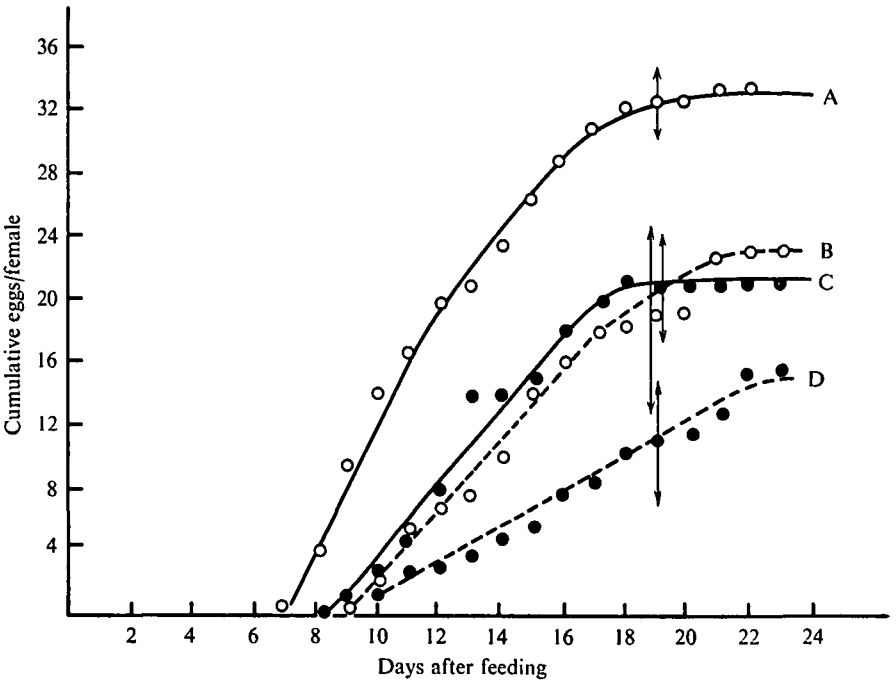
Text-fig. 8. The effect of topically applied FME on egg production in virgin (solid circles, solid lines) and decapitated mated (open circles, dotted lines) females. Arrows show 95 % fiducial limits.



Text-fig. 9. The effect of graded doses of FME on the rate of oviposition and on total egg production in virgin females. Each point represents the mean of the egg production of at least seven females, and the numbers opposite each point represent the dose in μl of FME applied. The parameters for a group of mated animals not treated with FME are also included.

dose required to produce the maximum juvenilizing effect on moulting 5th instar *Rhodnius* (Wigglesworth, 1969; Pratt & Davey, unpublished data).

In addition to an effect on egg production, a second important effect – on the rate of oviposition – is also demonstrable in these experiments. Text-fig. 9 relates the total egg production to the number of eggs laid by day 14 (an index of the rate of oviposition) for each of the levels of FME employed. The dose–response relationships for the two parameters are closely similar.



Text-fig. 10. The effect of FME on the rate of oviposition in females lacking their neurosecretory cells. A, Sham-operated, mated females treated with solvent only; B, operated females treated with 0·5 μl of FME; C, operated females treated with solvent only; D, operated females treated with 0·05 μl of FME. Each point represents the mean of those animals which eventually oviposited, and the arrows on the points for day 19 indicate the extent of the standard errors.

Since the neurosecretory cells of the pars intercerebralis are known to be involved in the rate of oviposition (Davey, 1967), and since high doses of FME are known to stimulate neurosecretion in nematodes (Davey, 1971), the effect of FME upon oviposition was determined in animals lacking their neurosecretory cells. In these experiments the females were operated upon (Davey, 1967) immediately after the first adult meal, and the FME was applied on the fifth day after the meal. The results appear in Text-fig. 10 and demonstrate that removal of the neurosecretory cells abolishes the effect of FME upon rate of oviposition. This experiment has been repeated twice with identical results. In none of these experiments did the total number of eggs produced by the females lacking their neurosecretory cells and treated with FME approach the number of eggs produced by a mated female.

DISCUSSION

The paper is concerned with the mechanism by which a failure to mate produces a depressed rate of egg production so that a virgin female produces about two-thirds as many eggs as a mated female. Previous work has shown that a blood-borne factor from mated spermathecae constitutes the signal of 'matedness' in the female, and that the neurosecretory cells of the pars intercerebralis are also involved in the process (Davey, 1965, 1967). A mated female not only lays more eggs, but she begins to oviposit earlier in the cycle at a higher rate than a virgin female.

However, the circumstances in which the neurosecretion is required in order to stimulate egg production require careful definition. In his studies, Coles (1965) noted the dramatic effect of virginity upon egg-laying, but his measurements of ovarian dry weights during what constitutes only the first gonotrophic cycle led him to propose that there was no significant effect of mating upon egg production. We have confirmed his observations directly in an earlier paper (Pratt & Davey, 1972*b*) and in the present paper we have described the growth of the ovaries in terms of the development of the individual oocytes. This extended analysis shows quite clearly that the effect of mating is limited to the second wave of oocytes. Is the neurosecretion then required only for the second wave of oocytes?

The fact that mated animals lacking their neurosecretory cells produce approximately the same number of eggs as unoperated virgins when observed over a number of consecutive feeding cycles (Davey, 1967) appears to establish that neurosecretion is not required for the development of the first wave of oocytes. Accordingly, we propose that the role of neurosecretion in permitting the full expression of the reproductive potential of this animal does not involve a direct trophic action of the neurosecretion upon the oocytes because we reject the notion that successive waves of oocytes have different intrinsic endocrine requirements.

Faced with a lack of direct action of neurosecretion upon the oocytes, it was natural to explore the only known alternative endocrine source – the corpus allatum. Despite the evidence suggesting that the juvenile hormone did not mediate the increase in egg production following mating (Davey, 1967), we further investigated the characteristics of virgin inhibition with this hypothesis in mind. An examination of the development of the ovaries during the second cycle of egg production when virgin inhibition is manifest did indeed reveal symptoms typical of surgical allatectomy. In brief, there was a typical accumulation of younger oocytes in the critical activation size range of 300–400 μm and a depression in the rate of vitellogenesis of those oocytes already activated, which resembled and even exceeded that observed following surgical allatectomy (Pratt & Davey, 1972*a*). The depression of vitellogenesis is accompanied by a reduction in patency of the follicular epithelium which we regard as an adequate explanation for the slow rate of vitellogenesis.

Despite the apparent resemblance of the virgin condition to that obtaining after surgical allatectomy, we had no experimental evidence that juvenile hormone was no longer available to the ovary, or that this was the cause of the inhibition of ovarian development. Accordingly we endeavoured to replace the supposed absence of juvenile hormone by the topical application of its analogue, FME. The chemical identity

of the authentic hormone in *Rhodnius* is not known, but FME is known to possess a high potency in this species as a juvenilizing agent and as a gonadotropin (Wigglesworth, 1963). We have assumed that FME simulates the authentic hormone in all its important trophic actions. First of all we established the dose of FME required to elicit maximal egg production in decapitated mated females. The dose required and the number of eggs produced are in good agreement with the figures obtained by Wigglesworth (1963). We then focused attention upon the fact that the number of eggs thus produced was only that characteristic of the number produced by a virgin female, that is to say about two-thirds of the egg production of a normal mated female. In view of this quantitative similarity we have proposed that the failure to achieve maximal egg production by hormone replacement in decapitated females was a meaningful observation related to the incompetence of the decapitated female to respond to the mating stimulus.

Physiological doses of FME applied to intact virgins produce no increase in the number of eggs produced. In order to achieve the egg production characteristic of mated animals, it was necessary to apply at least 500 nl of FME, a dose which is 100 times that required to produce complete juvenilization of moulting 5th instar larvae and at least 50 times that required to promote ripening of the first wave of oocytes in decapitated mated females.

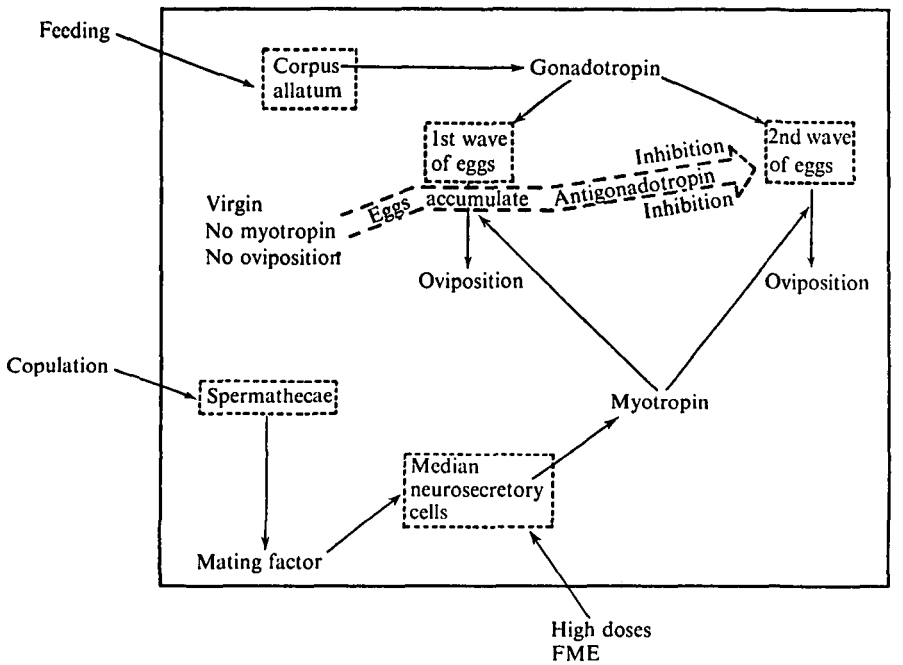
Large doses of FME and juvenile hormone are known to promote neurosecretory activity in the nematode *Phocanema decipiens* (Davey, 1971). We therefore tested the hypothesis that high doses in virgin *Rhodnius* substitute for the stimulus of mating by triggering an unnatural release of the neurosecretory myotropin. Evidence pertinent to this hypothesis emerged from observations on intact virgins which showed a proportional ability of applied FME both to increase egg production and to accelerate oviposition. Direct confirmation was obtained by applying FME to virgins from which the neurosecretory cells had been removed. In these experiments even the highest dose of FME failed to stimulate oviposition. Therefore the correlation between egg production and oviposition induced by FME in intact animals is not the result of a direct action of FME on the process of oviposition, but requires an intact neurosecretory system. Furthermore, as in decapitated females, the total numbers of eggs produced by a virgin female lacking her neurosecretory cells could not be elevated to the mated level by FME.

The intact virginal female, the female lacking neurosecretory cells and the decapitated female all have in common the inability to produce the mated level of eggs in the presence of a physiological dose of FME. These three categories of female also have in common the partial or complete absence of oviposition during the second half of the ovarian growth cycle. It is therefore reasonable to suggest that the retention of mature eggs in the female reproductive tract in some way contributes to this inhibition. These findings seem to be consistent with two types of explanation: either that some part of the reproductive tract of the virgin female releases a material which blocks those ovarian mechanisms which would otherwise respond positively to juvenile hormone, or else that the tract removes from the haemolymph circulation some as yet unidentified co-operative factor which is necessary for the expression of the response to juvenile hormone.

We emphasize that there is nothing in the above data to discriminate unequivocally

between these two hypotheses; rather, it is on the basis of intuition and precedent that we select the first as being the most plausible.

The suggestion that an 'antigonadotropin' arising as a consequence of the retention of mature oocytes or eggs in the female tract, might be involved in the control of egg production has been put forth in various forms by other workers. Nayar (1958) invoked a hormone emanating from the mature ovaries of the bug *Iphita limbata*, which was thought to inhibit the corpus allatum via the neurosecretory system.



Text-fig. 11. The network of influences believed to affect egg production in *Rhodnius*. The space enclosed by the large rectangle represents the insect and the spaces enclosed by the broken lines represent various anatomical structures within the insect.

Adams and his colleagues (Adams, Hintz & Pomonis, 1968; Adams, 1970) have described an 'oostatic hormone' from the ovaries of *Musca domestica* which is believed to inhibit the secretion of the corpus allatum. It has also been shown that the inhibition of egg development in pregnant ovoviviparous cockroaches is a consequence of the presence of the ootheca in the reproductive ducts. It has been suggested (Engelmann, 1965) that the walls of the pregnant reproductive tract release a humoral mediator which inhibits the corpus allatum via the central nervous system.

These observations on other insects notwithstanding, we have failed to find any evidence consistent with the hypothesis that it is the rate of release of the gonadotropin from the corpus allatum of *Rhodnius* which is controlled by our proposed antigonadotropin. Indeed, our evidence shows that the virgin female is incapable of responding to physiological doses of FME.

The complex network of stimuli, endocrine messengers and target organs which we believe to operate during the cycle of egg production in *Rhodnius* is displayed in the accompanying diagram (Text-fig. 11).

The antigonadotropin could act by competing with the gonadotropin for target sites in the follicle or by preventing the appearance of these sites. Alternatively, the antigonadotropin could inhibit some other processes downstream from the site of action of the gonadotropin. In this case the ovarioles would acknowledge the arrival of the gonadotropin but would be unable to express their activation because of a functional block in part of the machinery required for vitellogenesis. We have presented evidence that an important gonadotrophic function of the corpus allatum is to stimulate the appearance of intercellular spaces in the follicular epithelium (Pratt & Davey, 1972*a*). In the second wave of egg production in virgins, many of these spaces in large vitellogenic follicles become occluded. If this is a result of the direct action of the antigonadotropin upon the follicle it would seem that this factor must interfere with some process lying between the receptor site for the gonadotropin and the cellular machinery which brings about the morphological change in the epithelium.

We have considered the possibility that the antigonadotropin may block vitellogenesis by inhibiting the extra-ovarian synthesis of some essential metabolite or yolk precursor. The most likely candidate for such a precursor is the yolk protein itself. However, our observations showed that, rather than declining with the onset of virgin inhibition, the level of yolk protein in the haemolymph remains high and indeed persists at high levels for longer than it does in mated females. These results, besides showing that the titre of yolk protein is not a rate-limiting factor in virgin inhibition, provide a simple demonstration that *Rhodnius* is capable of controlling the level of yolk protein in its haemolymph independently of the control of vitellogenic activity in its ovaries.

SUMMARY

1. Mated females lay more eggs than virgin females. They also oviposit earlier in the cycle, and at a higher rate, than virgins.

2. Mated females produce slightly more than two oocytes per ovariole. Virgin females produce a little more than one oocyte per ovariole. The first wave of oogenesis proceeds at the same rate in mated and virgin females, whereas the second wave is inhibited in virgins.

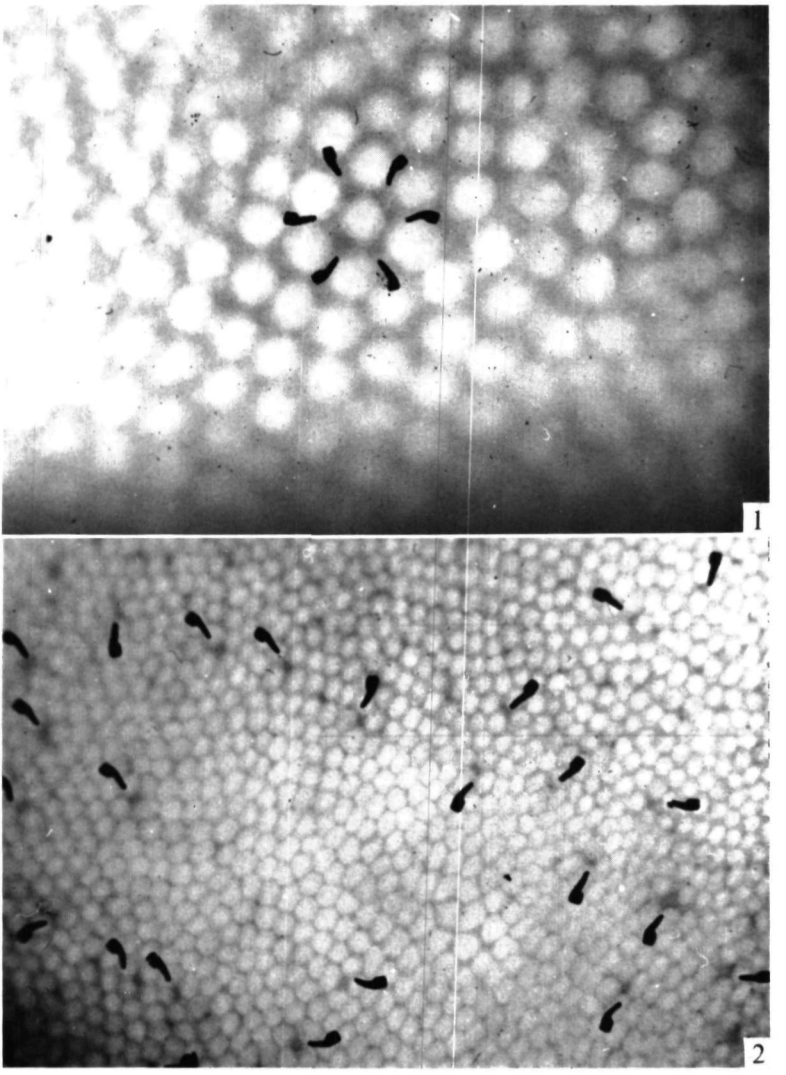
3. The concentration of yolk protein in the haemolymph remains high for longer in virgins than in mated females. The inhibition of egg production during the second wave of oogenesis in virgins is thus not a consequence of a previous decline in yolk-protein titre.

4. Virgin females digest their blood meal more slowly than mated females.

5. The follicular epithelium of an oocyte in the second wave of oogenesis in a virgin shows sparsely scattered intercellular spaces, whereas that of an oocyte from the first wave exhibits the abundant spaces characteristic of a follicle from a mated female.

6. During the time when vitellogenesis is inhibited in virgins there is an accumulation of oocytes in the size range below that of activation.

7. The corpora allata of both mated and virgin females undergo a cycle of increase in size associated with the cycle of oogenesis. There are no differences which can be correlated with the differences in the activities of the ovaries of mated and virgin females.


1
2

8. Topical application of farnesyl methyl ether (FME) to decapitated mated females brings about egg development equivalent to that of virgin females.

9. Topical application of FME to virgin females results in the production of the number of eggs characteristic of mated females, but very high doses are necessary to achieve this. These dose levels also result in an increase in rate of oviposition. These effects of high doses of FME are abolished if the virgin females lack their neurosecretory cells.

10. It is concluded that the virgin inhibition of oogenesis results not from a deficiency of allatum hormone, but from the presence of an antigonadotropin released from ovaries which contain mature eggs.

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EXPLANATION OF PLATE

Fig. 1. Surface view of a follicle from the first wave of oocyte production in a virgin female. The ovariole had been immersed in Evans blue just before the photograph was taken, and the dye has penetrated between all of the cells. The spaces around one cell are indicated by the comma-shaped markers. × 800.

Fig. 2. As in fig. 1, but showing a large follicle from the second wave of oocyte production in a virgin female. The spaces revealed by Evans blue are sparsely scattered over the surface of the follicle and some of these spaces are indicated by the comma-shaped markers. × 450.