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

II. THE EFFECTS OF STARVATION

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INTRODUCTION

In his classic examination of the function of the corpus allatum in *Rhodnius*, Wigglesworth (1936) reported that during starvation there is a resorption of partially developed eggs. This condition is associated with a shrunken corpus allatum, and animals allatectomized by decapitation have ovaries which resemble those of starved animals. Wigglesworth suggested that starvation and allatectomy were interrelated.

Quantitative studies in this laboratory have shown that surgical allatectomy reduces but does not abolish egg production in *Rhodnius* (Davey, 1967; Patchin & Davey, 1968). We have established a number of characteristics of the allatectomized animal (Pratt & Davey, 1972a), and the present paper examines the starved female with respect to these parameters.

MATERIALS AND METHODS

All of the experimental animals were maintained and mated as described previously (Pratt & Davey, 1972a). For these experiments on starvation, however, it was necessary to use insects in their second cycle of egg production. Mated females were fed within 7 to 10 days of the adult moult and were then kept without food for periods of 14, 21 or 28 days before re-feeding. Observations were thus made on the cycle of egg production which followed the second feeding. The response to longer periods of fasting was not examined because there was significant mortality after 28 days.

Because *Rhodnius* takes a large blood meal which it stores in the crop, animals can only be considered as 'starved' when these reserves are exhausted, or when food is unable to pass from the crop to the intestine. In a mated female the crop becomes empty on the twelfth day after feeding, and the periods of fasting used here are thus equivalent to 2, 9 and 16 days of starvation. A period of 2 days of starvation has no detectable effect upon the concentration of yolk protein in the haemolymph or upon ovarian development and it does not induce resorption. The control or 'normal' animals used in these experiments are thus kept without food for 14 days.

The other procedures employed in this study have been described earlier (Pratt & Davey, 1972a).

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RESULTS

Starvation and ovarian growth

An index of ovarian growth is the dry weight of the ovaries on successive days after feeding. Text-fig. 1 shows the dry weights of ovaries of mated females on each of the first six days after re-feeding subsequent to a period of fasting of 14, 21 or 28 days. There is an obvious delay in the resumption of ovarian growth which becomes greater with longer periods of starvation.
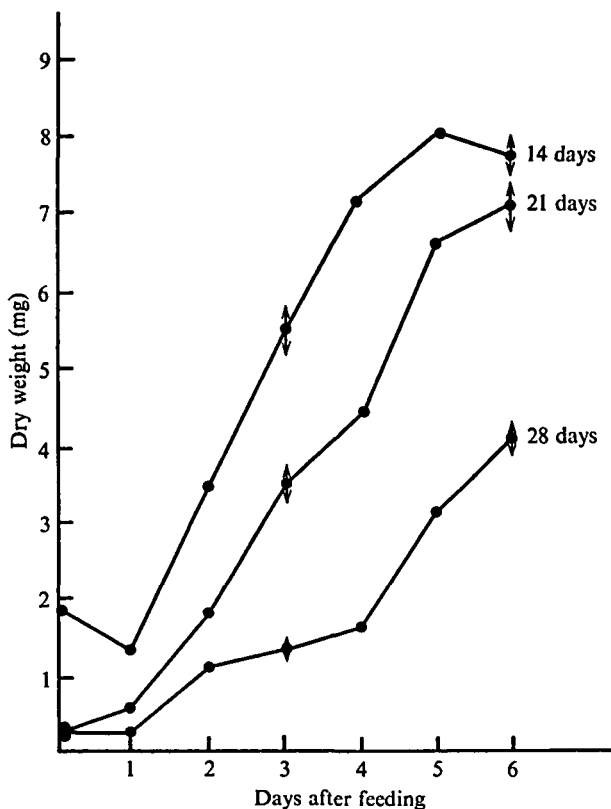
Text-fig. 1. Dry weights of ovaries on each of the first 6 days after re-feeding subsequent to a period of fasting of 14, 21 or 28 days. Each point indicates the mean of the determinations for four to eight females. The arrows at 3 and 6 days indicate the standard errors of the means.

### *Starvation and the concentration of yolk protein*

Text-fig. 2 shows the equivalent concentration in the haemolymph of yolk antigen on each of the first 6 days after re-feeding mated females which had been starved for 16 days. The range of 'normal' concentrations, obtained from animals which had fasted for 14 days (2 days of starvation) has been included for comparison. Note that although the level of yolk protein is very low following a period of starvation, it recovers quickly and becomes normal by day 3. Furthermore, the exsanguination volume of the starved females before re-feeding is less than 2 μl, but within one day

f re-feeding the volume of haemolymph obtainable from a female has risen to over 20μl, suggesting a period of active synthesis.

Text-fig. 2 also includes a measure of vitellogenesis expressed as the percentage of ovarioles which contain oocytes greater than 400 μm in length. Oocytes of this size range are known to be vitellogenic (Pratt & Davey, 1972a). Thus, in a starved, re-fed female, the resumption of vitellogenesis is delayed until 4–5 days after the meal, whereas the synthesis and/or release of the yolk protein is sufficiently great in the first days following the meal to return the concentration to the normal level by the third day after feeding.

Starvation and the size of oocytes

The terminal oocytes in ovarioles removed from mated females on successive days after re-feeding subsequent to 16 days of starvation were measured. Text-fig. 3 plots the percentage of terminal (T) oocytes in each of three size classes for these ovarioles. The details of the rationale underlying the choice of these size classes may be found in the previous paper (Pratt & Davey, 1972a). Briefly, oocytes of lengths between
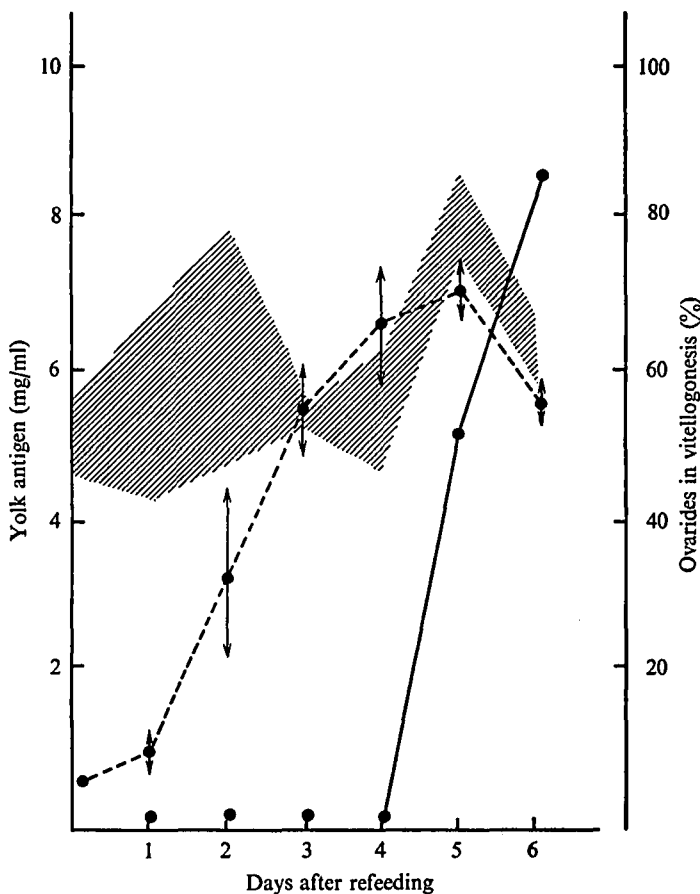
Text-fig. 2. The effect of 28 days fasting on the level of yolk protein in the haemolymph (broken line) on each of the first six days after re-feeding. The arrows indicate the standard errors. The shaded portion represents the level of yolk protein (mean ±S.E.) in animals which have not been starved. The solid line represents the percentage of ovarioles containing vitellogenic oocytes.

300 and 400 μm are in a critical size range capable of activation, a phenomenon which precedes vitellogenesis and which is signalled by the patency of the follicular epithelium surrounding the oocyte. Those below that range are incapable of activation, while those larger than 400 μm are in active vitellogenesis.
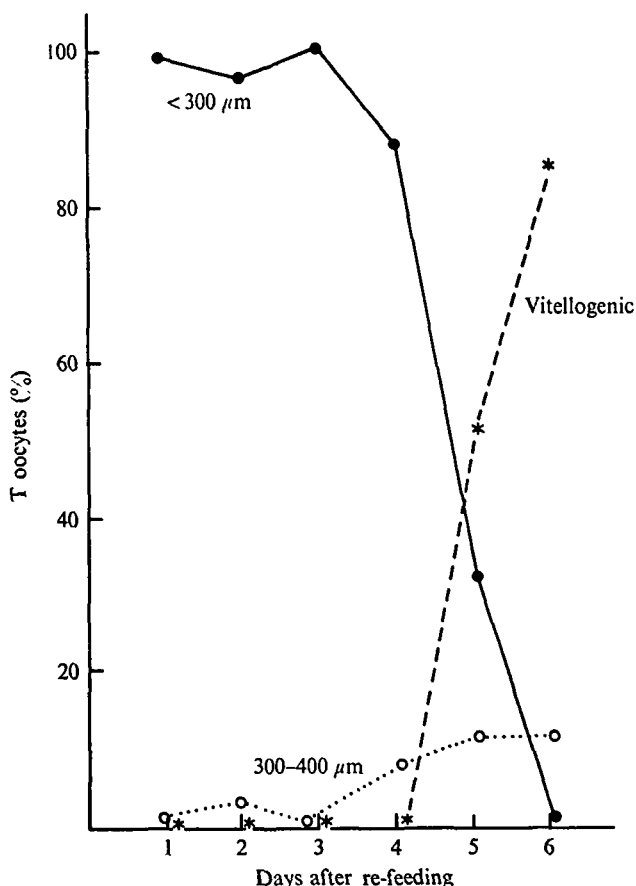
Text-fig. 3. The percentage of terminal (T) oocytes in various size classes in ovarioles removed from females at various times after re-feeding subsequent to a period of fasting of 28 days.

From Text-fig. 3 it is clear that starvation induces an accumulation of oocytes in the range below that of activation until vitellogenesis begins on the fourth and fifth days after re-feeding. At that time there is a rapid decrease in the percentage of terminal oocytes in that size range without a compensating increase in the next largest size range. Thus the terminal oocytes pass rapidly through the critical activation size about 4 days after feeding and enter vitellogenesis. A comparison of this figure with Text-fig. 6 in the previous paper (Pratt & Davey, 1972a) reveals that the 'starved' ovariole does not resemble the 'allatectomized ovariole. Allatectomy results in an accumulation of oocytes in the critical 300–400 μm size range rather than in the pre-activation stages In the 'starved' ovariole, the terminal oocytes immediately after re-feeding had an average length of only 150 μm.

Starvation and the tropharium

A normal tropharium on the third day after feeding stains deeply with Ponceau S (Pl. 1, fig. 1), as does the tropharium from an allatectomized animal (Pl. 1, fig. 2). However, the tropharium from a female starved for 16 days shows scarcely any staining even on the third day after re-feeding (Pl. 1, fig. 3). At the same time enlarged trophocytes with their characteristic inclusions are totally absent from the ovarioles of starved females during the first 6 days after re-feeding (Pl. 1, fig. 4). By contrast, the tropharia of normal and allatectomized females contain up to 40 such cells on the third and fourth days after feeding (Pratt & Davey, 1972a).

DISCUSSION

The data presented in this paper relate only to two questions. Is the starved, re-fed animal suffering from physiological allatectomy and, if so, is this what is responsible for the delay in vitellogenesis? Does the level of yolk protein in the haemolymph regulate the rate of vitellogenesis in the intact animal?

We have no information on whether the starved re-fed animal has or has not high titres of circulating juvenile hormone in its haemolymph. However, such information appears to be irrelevant in the face of the observation that the ovarioles of such animals display none of the symptoms which we have shown to be diagnostic of an ovary deprived of its juvenile hormone by surgical allatectomy (Pratt & Davey, 1972a). On the other hand, the 'starved' ovariole displays symptoms such as protein attrition and apparent synthetic disability in the tropharium for which we can ascribe no known endocrine cause. While it may be true, as Wigglesworth (1936) suggested, that pre-vitellogenic growth continues during starvation, we have not found that such pre-vitellogenic growth ensures the continued presence of oocytes in the size range capable of responding immediately to juvenile hormone. We thus agree with the observations of Danilov (1967), who has found that resorption of the pre-vitellogenic oocytes occurs after prolonged starvation in *Rhodnius*.

The second question arises in the context of the conclusion drawn by Coles (1965) and Vanderberg (1963) that the juvenile hormone controls both yolk deposition in the ovaries and the synthesis of the protein which constitutes the raw material for the yolk. We have confirmed in an earlier paper that surgical allatectomy results in a lowered concentration of yolk protein in the haemolymph (Pratt & Davey, 1972a). This evidence is, in fact, more convincing than that of Coles (1965), who has shown that there is no accumulation of yolk protein in the blood of females whose ovaries have been inhibited by decapitation.

Nevertheless, neither our results nor those of Coles (1965) constitute unequivocal proof that the intact animals regulate vitellogenesis by means of the level of the yolk protein in the haemolymph. In the present paper we have examined the level of the yolk protein under conditions of starvation when vitellogenesis is inhibited and we have made the disappointing discovery that during all of the time when the yolk protein level is low, and for part of the time when it is high, the oocytes are incompetent to respond to the gonadotropin because of their diminutive size. Thus, whether or not the early re-establishment of the normal level of yolk protein in the haemolymph

occurs in the presence or absence of juvenile hormone, its increased level anticipates the demand for it by the oocytes. In the starved or recently re-fed animal it is not the level of yolk protein in the haemolymph which limits vitellogenesis.

In the next paper in this series (Pratt & Davey, 1972b) we will examine the levels of yolk protein in the virgin and in the mated female. These results will demonstrate that the level of yolk protein remains high for some time after vitellogenesis has ceased, particularly in virgins, even when oocytes (according to their size range) should be capable of responding to the gonadotropin by taking up the available yolk protein. We have therefore failed to find any circumstance in the intact animal in which the concentration of yolk protein in the haemolymph could be a rate-limiting factor in vitellogenesis.

SUMMARY

1. In females which have been re-fed after various periods of fasting there is a delay in the resumption of ovarian growth which becomes greater with longer periods of starvation.

2. In females kept without food for 28 days before re-feeding, vitellogenesis is not resumed until 4 or 5 days after the meal, whereas the concentration of yolk antigen in the haemolymph returns to its normal level by the third day after the meal.

3. A period of starvation induces an accumulation of oocytes in the size range below that of activation until the fourth or fifth day after re-feeding, when the terminal oocytes pass rapidly through the critical size range and enter vitellogenesis.

4. The tropharia of starved females exhibit a remarkably reduced affinity for the protein stain Ponceau S, and enlarged trophocytes with their characteristic inclusions are absent during the first 6 days after re-feeding.

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Plate 1

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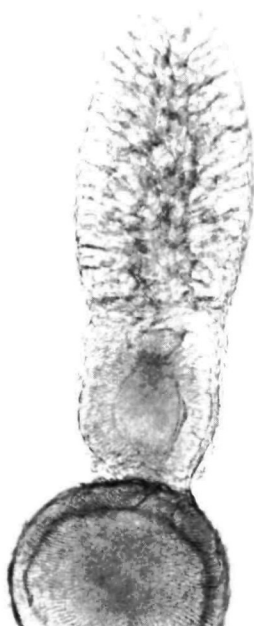
EXPLANATION OF PLATE

Fig. 1. Whole mount of a tropharium from a normal female on the third day after feeding. The tropharium, which has been stained with Ponceau S to reveal protein, is deeply stained, and the spheres indicative of active trophocytes are clearly visible. × 120.

Fig. 2. As in fig. 1, but from an allatectomized female. × 120.

Fig. 3. As in fig. 1, but from a female kept without food for 28 days before re-feeding. Note that even on the third day after re-feeding the tropharium fails to stain for protein. × 120.

Fig. 4. A tropharium from a female kept without food for 28 days and killed on the sixth day after re-feeding. Note that although the protein staining is now evident there are no spheres, indicating that the trophocytes are inactive. × 120.