

THE ACTION OF JUVENILE HORMONE ON THE FOLLICLE CELLS OF *RHODNIUS PROLIXUS*: THE IMPORTANCE OF VOLUME CHANGES*

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SUMMARY

1. The onset of vitellogenesis in *Rhodnius prolixus* is marked by a reduction in the height of the follicle cells. This decrease is not observed in follicle cells from allatectomized females. Estimates of the changes in cross-sectional area of the cells suggest that the cells shrink to about 50% of their original volume as the result of JH action.

2. Determination by interference microscopy of the volume of isolated living follicle cells before and after exposure to JH also suggest that the volume is reduced to 50% of the original volume as a result of JH action. There was no decrease in volume of follicle cells from allatectomized females following exposure to JH.

3. During mid to late vitellogenesis *in vivo*, an increase in cell volume was measured, an increase that possibly reflects an increase in cellular dry mass.

4. It is concluded that follicle cells normally respond to JH by pumping out fluid, thus reducing their volume, and leading to the development of spaces between the cells.

INTRODUCTION

An important effect of juvenile hormone (JH) in the adult female of *Rhodnius* involves the induction of large spaces between the cells of the follicular epithelium surrounding the oocytes, thus providing a route for the passage of extra-ovarian yolk proteins from the haemolymph to the oocyte surface (Pratt and Davey, 1972). This response of the follicle cells to JH has been demonstrated in ovaries maintained for short periods *in vitro* (Davey & Huebner, 1974).

Such a response on the part of the follicle cells can result from a change in the cytoskeletal elements (producing an increase in the height of the cells and a corresponding decrease in their diameter) or from a decrease in the volume of the cells (provided that their height does not proportionately decrease), or from some combination of these two phenomena. Both mechanisms will require an intact cytoskeletal system, and it is therefore hardly surprising that disrupters of cytoskeletal integrity inhibit the response of the cells to JH (Abu-Hakima, 1976). These observations, however, do not demonstrate that changes in the cytoskeleton are important to the response.

The present study investigates two crucial questions. Is there evidence of elongation of the follicle cells during vitellogenesis? Do follicle cells reduce their volume when

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exposed to JH? Two approaches are employed. Firstly, the relevant morphometric of the follicle cells during oocyte growth are defined, thereby providing information about changes in the dimensions of the cells as they respond to JH *in vivo*. Secondly, interference microscopy is employed to measure possible changes in volume of living isolated follicle cells exposed to JH.

We have reported previously that the follicle cells of the allatectomized mated female are unable to respond to JH *in vitro* (Abu-Hakima & Davey, 1975). This phenomenon is examined further in the light of what is now known about the action of JH on follicle cells from normal females.

MATERIALS AND METHODS

Experimental animals were taken from a colony maintained at 28 °C and at high relative humidity, and consisted of normal mated females and mated females which had been allatectomized as described in previous reports from this laboratory (Abu-Hakima & Davey, 1975; Pratt & Davey, 1972).

Morphometric studies

Whole mounts of ovarioles from normal mated or allatectomized mated females were stained in Ponceau-S as described by Pratt & Davey (1972). Ovarioles which were embedded in epoxy resins for electron microscopy were also used. Material of both types had been fixed in glutaraldehyde at various times after the females had been fed; this procedure produces oocytes in different stages of development as defined by their size (cf. Pratt & Davey, 1972). The follicular dimensions were measured with an ocular micrometer, using a 10 × objective. The height of two lateral follicle cells was measured in each follicle. This parameter was remarkably uniform for the cells within a particular follicle.

The surface area of the oocyte was calculated from the formula $12.57r^2$ in the case of spherical oocytes. After the oocyte had begun to elongate the following formula was used:

$$6.28ab \left(\frac{\sin^{-1} \epsilon}{\epsilon} + \sqrt{(1 - \epsilon)^2} \right),$$

where a = major radius, b = minor radius, and $\epsilon = \sqrt{[1 - (b^2/a^2)]}$.

Isolation of follicle cells

The follicle cells examined by interference microscopy were isolated from vitellogenic follicles of either normal or allatectomized mated females. Ovaries were dissected from the insects and placed in culture medium (Davey & Huebner, 1974). Ovarioles were separated from the surrounding sheaths, and the lateral follicle cells of vitellogenic oocytes were separated from the rest of the ovariole using electrolytically sharpened needles. It is important to avoid the follicle cells at the anterior and posterior ends of the follicles since spaces do not appear between these cells. Gentle pipetting in and out of a finely drawn Pasteur pipette is sufficient to free the follicle cells from the remainder of the oocyte and from one another. Preparations containing very little debris and consisting of single cells, or of no more than ten cells, could,

obtained in this way. The cells were accumulated in fresh culture medium in small glass vials.

Interference microscopy

Measurements were made using the 63 × objective on an 'Interphako' microscope (Jena Instruments). Specimens were illuminated with monochromatic light (546 nm) using an HBO 200 mercury-arc lamp. By measuring the optical path of the medium alone and of the cell immersed in the medium, the optical path difference, o.p.d., between the cell and the background can be calculated. For each cell, mean values of o.p.d. were determined from a series of alternate measurements of the cell and surrounding medium. The area of some of the cells was measured from photographs using a planimeter.

From the values of o.p.d., certain parameters can be calculated, according to the following equations:

$$C = \frac{n_0 - n_m}{\chi}, \quad (1)$$

where C = concentration (weight of cell solids per unit volume), n_0 and n_m = refractive index of the cell and the medium respectively, and χ = specific refractive increment, which can be taken as a constant (Davies, 1958). The o.p.d. can be expressed as:

$$\text{o.p.d.} = (n_0 - n_m) t, \quad (2)$$

where t = thickness of the cell (Davies, 1958). Equation (1) can thus be expressed as:

$$C = \frac{\text{o.p.d.}}{\chi t}. \quad (3)$$

Volume and concentration are inversely related, and the ratio between the concentration of a cell in medium alone (C_M) and in JH (C_{JH}) can be expressed in terms of volume (V_M and V_{JH} , respectively). If it is assumed that t does not change, then,

$$\frac{V_{JH}}{V_M} = \frac{C_M}{C_{JH}} = \frac{\text{o.p.d. in medium alone}}{\text{o.p.d. in medium + JH}}. \quad (4)$$

This equation also assumes that changes in C are not due to changes in dry mass of the cell. This assumption is discussed later.

The JH used in these experiments was a mixture of the stereoisomers of C_{18} JH (JH 1), and was a gift from Ayerst Laboratories, Montreal. It was used in the medium at 10^{-4} $\mu\text{l/ml}$.

RESULTS

Morphometrics of the follicle during early vitellogenesis

Fig. 1 displays the mean follicle cell height for each 100 μm interval of oocyte length. The first three intervals (up to 400 μm) represent previtellogenic growth, intervals 4–15 (up to 1600 μm) represent vitellogenic growth, and intervals 16 and 17 postvitellogenesis and chorion formation. It is clear from Fig. 1 that changes in the height of the follicle cells occurred in follicles from both normal and allatectomized females. In the normal female, the follicle cells decrease in height by about 20%

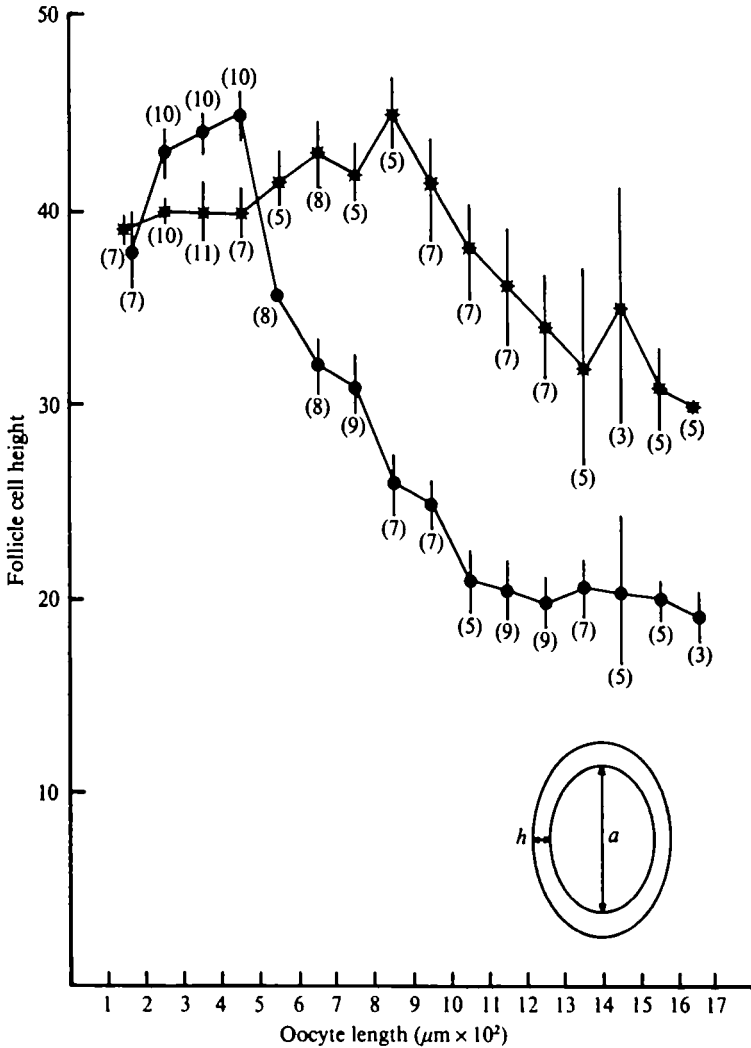


Fig. 1. Mean changes in the follicle cell height during egg development in normal (\bullet) and allatectomized (\star) females. Bars represent \pm s.e.

during the earliest stage of vitellogenesis (between intervals 4 and 5). In the allatectomized female, however, there was an increase of some 4% over the same interval. Changes in follicle cell height later in vitellogenesis will be dealt with below. The finding that the follicle cells decrease markedly in height at 'activation' (cf. Pratt & Davey, 1972) between intervals 4 and 5 in normal females, and that this decrease does not occur in allatectomized females, argues strongly that the decrease is a result of JH action. It also constitutes a powerful argument against the hypothesis that patency of the follicular epithelium is a consequence of changes in shape of the cells.

A calculation of any change in volume of the cells requires knowledge of the change in cross-sectional area of the cells as a result of JH action. An estimate of this can be obtained from photographs of vitellogenic follicles exhibiting a maximum patency index in the Evans' Blue dye penetration test (Pratt & Davey, 1972; Davey & Huebner

1974). By weighing such photographs before and after the follicle cells had been cut out, it was estimated that 30% of the total cross-sectional area was occupied by interfollicular cell space. Since the appearance of the spaces is a result of JH action, this implies that the area of a follicle cell decreases to 70% of its original value as a result of JH action.

If we also assume that the change in height between intervals 4 and 5 is due entirely to JH action, then

$$\begin{aligned} \frac{\text{volume after JH}}{\text{volume before JH}} &= \frac{\text{height after JH}}{\text{height before JH}} \times \frac{\text{area after JH}}{\text{area before JH}} \\ &= 0.8 \qquad \qquad \times 0.7 \\ &= 0.56 \end{aligned}$$

Thus, these measurements suggest that the follicle cells decrease in volume to about 56% of the preactivation volume as a result of JH action.

The above determinations represent a combination of measurements made on fixed tissues from *in vivo* material and measurements made on fresh tissues from *in vitro* material and are thus open to criticism. While the changes in surface area are ascribable exclusively to JH, the observed changes in follicle cell height may be blurred by other events. It became important, therefore, to use an approach where living follicle cells could be examined for possible changes in volume resulting from exposure to JH.

Volume changes in isolated follicle cells of mated females

Table 1 sets out the mean values of o.p.d. for at least 10 consecutive determinations in each of 14 different cells exposed to medium alone followed by exposure to the same cell to JH in medium at a concentration of 10^{-4} $\mu\text{l/ml}$. Each cell was from a different follicle.

Clearly, JH brings about an increase in o.p.d. between the cell and the background. While the magnitude of the change varied from cell to cell, the direction of the change was consistent, there always being an increase when the cell was exposed to JH.

In two cases, it was also possible to flush out the medium containing JH with fresh medium without losing track of the particular cell under observation. In such cases (see cells 9 and 12), the o.p.d. decreased, thereby demonstrating the reversibility of the effect of JH.

The increase in o.p.d. occurred rapidly upon exposure of the cells to JH. The time-course of the increase is depicted in Fig. 2, which demonstrates that the increase was apparent within 5 min, the earliest time at which measurements could be made. The data in Fig. 2 were obtained from only a few cells, and should be interpreted with caution. Nevertheless, the increase in o.p.d. is complete within 15 min of exposure to JH.

Concomitant with the changes in o.p.d., changes in area of the cells were sometimes noted. It was difficult to obtain satisfactory photographs for area determinations of every cell, but the available data are presented in Table 2. There was a slight decrease in the area of each of the cells after exposure to JH.

The values of o.p.d. can be used to calculate the ratio of the volumes of the cell before and after treatment with the hormone (see equation 4). For the calculations

Table 1. *Optical path difference of isolated follicle cells from normal mated females*

Cell	Mean o.p.d. \pm s.e.			$\frac{V_{JH}}{V_M}$
	In medium	In JH	After rinsing	
1	48.10 \pm 2.0	59.8 \pm 3.0	—	0.80
2	39.43 \pm 1.5	78.00 \pm 3.0	—	0.51
3	39.52 \pm 1.6	61.10 \pm 1.8	—	0.65
4	35.32 \pm 3.0	54.48 \pm 4.0	—	0.65
5	30.94 \pm 1.0	55.90 \pm 4.0	—	0.55
6	28.88 \pm 0.8	71.24 \pm 1.4	—	0.41
7	32.85 \pm 1.2	84.50 \pm 1.5	—	0.39
8	35.36 \pm 1.8	73.32 \pm 3.0	—	0.48
9	33.54 \pm 0.9	59.8 \pm 2.0	35.20 \pm 1.1	0.56
10	33.28 \pm 1.1	56.16 \pm 1.5	—	0.59
11	27.56 \pm 1.1	56.68 \pm 2.0	—	0.49
12	36.05 \pm 1.2	59.97 \pm 3.0	38.52 \pm 2.5	0.60
13	22.88 \pm 1.1	53.13 \pm 4.0	—	0.35
14	26.00 \pm 1.1	55.13 \pm 4.0	—	0.47

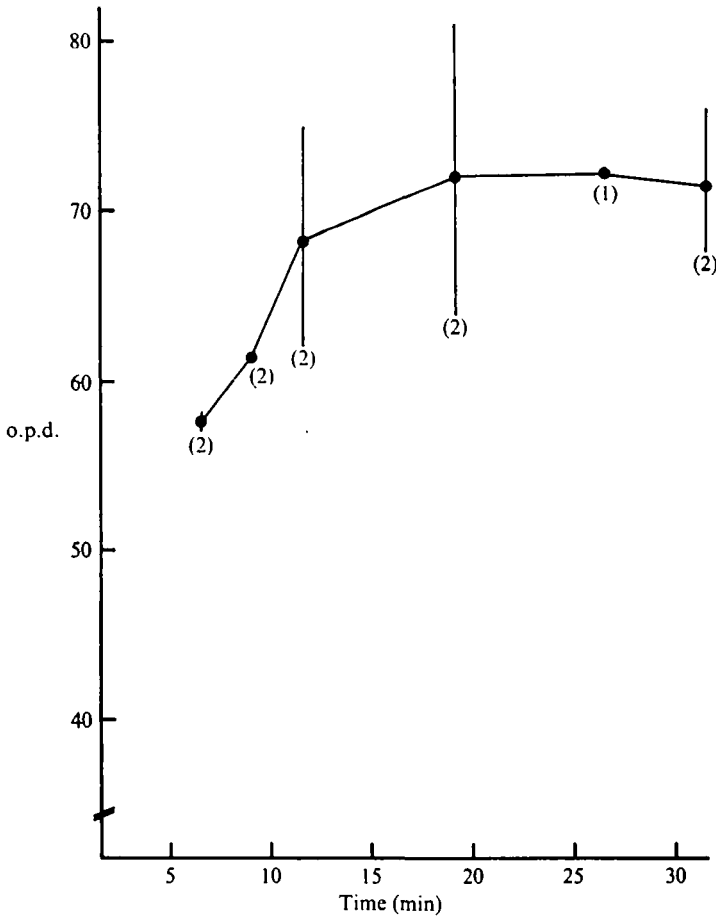
Fig. 2. Mean changes in o.p.d. of isolated follicle cells with time, in the presence of JH. Bars represent \pm s.e.

Table 2. Areas of isolated follicle cells from normal mated females

Cell	Measured area (cm ²)	
	In medium	In JH
9	6.69×10^{-4}	6.38×10^{-4}
11	1.90×10^{-4}	1.69×10^{-4}
12	5.00×10^{-4}	4.46×10^{-4}

we have had to assume that t does not change, for it proved to be technically impossible to determine t on a single cell in the presence and absence of JH. It should be noted that the parameter t refers to the optical path through an isolated follicle cell. As the general shape of the cells is cylindrical, in most cases t will represent the diameter of the cell. It is not equivalent to h , the height of the epithelium in an intact follicle. A second assumption is also necessary. The calculation of volume rests on the expression for concentration (see equation 3), which is defined as the dry mass of the cell per unit volume. An increase in concentration could thus be due to an increase in dry mass, resulting from net synthetic activity on the part of the cell. However, such an increase is unlikely to occur within the short time required for the full expression of the effect of JH, nor is such an increase likely to be readily reversible. The assumption that the increase in concentration results from a decrease in volume is probably justified. Table 1 therefore also sets out the value of the ratio $V_{\text{JH}}/V_{\text{M}}$ (see equation 4). The mean value of this ratio is 0.54, indicating that as a result of the application of JH, there was a decrease in volume of the individual cells to about 54% of the original volume.

It would be useful to know whether t in isolated cells does in fact decrease. Techniques exist for the determination of t by interference microscopy, but these require the determination of o.p.d. in two media of different osmotic concentration (Barer & Joseph, 1955*a, b*). It has not been possible to determine t by this double immersion procedure for the *same* cell in the presence and absence of JH. While a few determinations have been made for cells exposed to medium alone and for different cells exposed to medium plus JH, even this procedure has proved to be technically very difficult, and further attempts have been abandoned.

Another approach to the problem is possible. For three of the cells in Table 1, measurements of the area of the isolated cells in medium alone (I_{M}) and in medium plus JH (I_{JH}) are available (Table 2). Note that the parameter I refers to the area of the isolated cell; it is not necessarily equivalent to A , the cross-sectional area of the cells in the intact follicular epithelium. Now if t were not to change, then $V_{\text{JH}}/V_{\text{M}} = I_{\text{JH}}/I_{\text{M}}$. This is not the case, and since $I_{\text{JH}}/I_{\text{M}}$ is in each of the three cells greater than $V_{\text{JH}}/V_{\text{M}}$, then t must decrease in response to JH. The inference that t decreases does not alter the conclusion that the volume decreases to about 54% of its original value as the result of exposure to JH. It does, however, demonstrate that some part of the observed increase in o.p.d. is due to a decrease in t and is not wholly a result of an increase in C , the concentration of cell solids.

It is difficult to be certain about the relationship of the dimension t in isolated cells to a particular axis of the cells in the intact follicular epithelium. It is reasonable to assume, in view of the columnar nature of the epithelium, that t in an isolated cell is

Table 3. *Optical path difference of isolated follicle cells from allatectomized females*

Cell	Mean o.p.d. \pm S.E.		$\frac{V_{JH}}{V_M}$
	In medium	In JH	
1	26.69 \pm 0.6	24.26 \pm 0.6	1.10
2	25.48 \pm 0.9	22.88 \pm 0.5	1.11
3	30.69 \pm 0.6	33.28 \pm 0.6	0.92
4	34.84 \pm 0.8	35.88 \pm 0.9	0.97
5	36.40 \pm 1.0	33.80 \pm 1.1	1.08
6	23.92 \pm 1.0	24.96 \pm 1.0	0.96

represented in the intact epithelium by the diameter of the cell at right angles to its longitudinal axis. It should be emphasized that while the cells may move slightly during the changing of media, they do not roll over. Thus, for any one isolated cell, it is the same t which is measured in the presence and absence of JH.

In summary, the data from the morphometric measurements on fixed and living tissue at about the time that JH has its first effects suggests that the volume of the follicle cells decreases to about 56% of its pre-activation value. In experiments with isolated, living cells, the volume decreases to about 54% of the original level. There is thus a striking agreement between the values obtained by two very different methods.

Volume changes in follicle cells of allatectomized females

One of the obvious consequences of allatectomy is the anomalous behaviour of the follicular epithelium when compared with the normal female (Fig. 1; Abu-Hakima & Davey, 1975). Estimates of o.p.d. were therefore made in order to determine whether isolated follicle cells from allatectomized mated females responded to JH in a manner similar to cells removed from normal mated females.

Follicle cells isolated from vitellogenic follicles from allatectomized mated females failed to exhibit an increase in o.p.d. in response to JH (Table 3). The mean value of o.p.d. for six cells from allatectomized females was 29.18, comparing well with the mean value of o.p.d. of the same cells exposed to medium alone (29.71). Values for the ratio V_{JH}/V_M are also presented in Table 3, and demonstrate that there was no change in volume in response to JH, the mean value of V_{JH}/V_M being 1.02.

The morphometrics of post-activation growth

It has been argued above that the decrease in follicle cell height at the onset of vitellogenesis, between intervals 4 and 5 (Fig. 1), represents a response to JH. However, the follicle cells of the normal mated females (and to a lesser extent, of the allatectomized mated females) continue to decrease in height as vitellogenesis proceeds. What is the significance of this continued decrease in follicle cell height?

One possible explanation lies in the increase in the surface area of the oocyte. Fig. 3 relates the increase in the surface area to the stage of the oocyte. It is apparent that the increase in the surface area of the oocyte during egg development in both the normal and allatectomized female is very similar. This is predictable, since the oocytes of both grow to the maximum size, albeit at different rates (Pratt & Davey, 1972). It ■

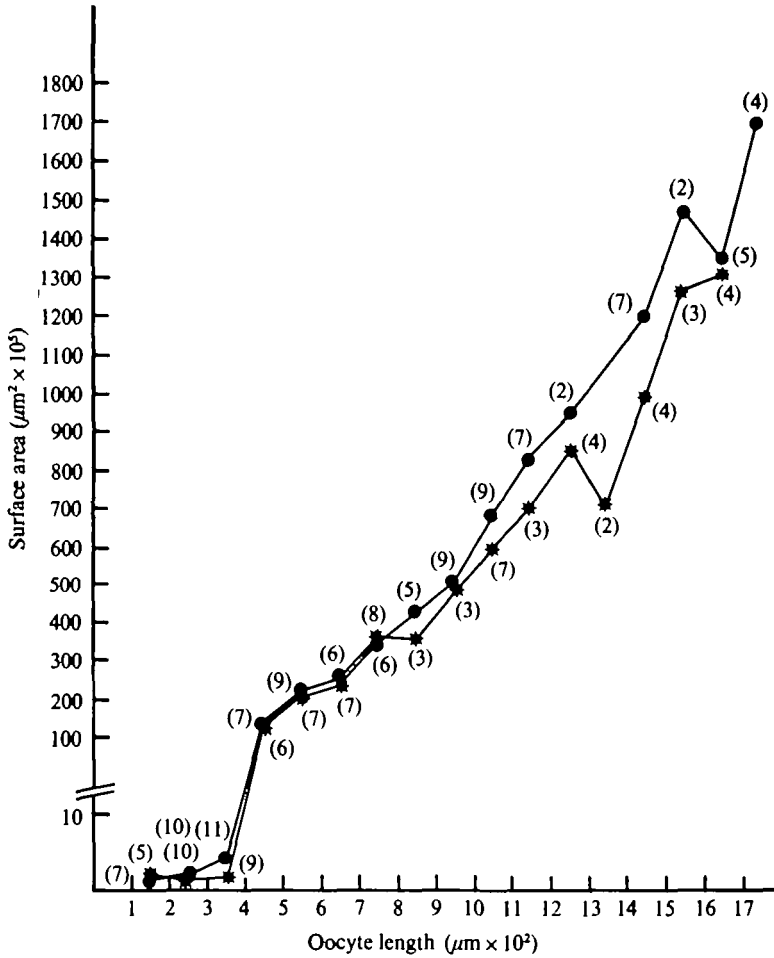


Fig. 3. Mean changes in the surface area of oocytes during egg development in normal (●) and allatectomized (★) females.

clear that after interval 5, the surface area increases in a linear fashion. On the other hand, the decrease in follicle cell height is not linear for the entire period of post activation growth. Thus, the continued decrease in follicle cell height might be explained by the increase in surface area of the oocyte as far as interval 10. However, between intervals 10 and 17, the cells fail to undergo a further reduction in height, while the surface area more than doubles. This suggests either an increase in volume of the cell, or an increase in the size of the spaces between the cells during this period.

Even for the period when the change in height is linear (between intervals 5-6 and 10-11), the decrease may not be simply a consequence of the increase in cell surface alone. Thus, the ratio:

$$\frac{\text{height at interval 5-6}}{\text{height at interval 10-11}} = 1.7.$$

The surface area of the follicle at interval 5-6 is $200 \times 10^5 \mu\text{m}^2$, of which 30% is assumed to be taken up by the spaces between the cells, and the area occupied by cells

Table 4. *Volumes of the follicular epithelium during vitellogenesis*

Size interval	Follicle cell volume ($\mu\text{m}^3 \times 10^5$)	
	Normal	Allatectomized
4	5 612	4 760
5	5 400	7 686
10	9 576	17 632
14	23 860	34 300
16	25 384	38 700

is therefore $140 \times 10^5 \mu\text{m}^3$. If the relative size of the intercellular space does not increase, then the surface area occupied by cells at interval 10-11 is $480 \times 10^5 \mu\text{m}^2$. Furthermore, if the volume of the cells does not change, then the following equation should be obtained:

$$V = A_5 \times h_5 = A_{10} \times h_{10},$$

and

$$\frac{h_5}{h_{10}} = \frac{A_{10}}{A_5},$$

where V = volume of cells in follicular layer, h_5 = height of cells at interval 5-6, h_{10} = height of cells at interval 10-11, A_5 = area of cells at interval 5-6, A_{10} = area of cells at interval 10-11. But $h_5/h_{10} = 1.7$ and $A_{10}/A_5 = 3.4$, and it is therefore clear that even for the period between intervals 5-6 and 10-11, where the decrease in height is approximately linear, both the volume of the cells and the size of the intercellular spaces may be varying.

To test the possibility that the relative size of the intercellular space may vary, follicles at intervals 5-6, or 10-11, were photographed from Ponceau-S stained whole mounts. From these photographs estimates of the total area occupied by spaces were obtained as described above. In the smaller follicles 26% of the surface area was found to consist of spaces, and for the larger follicles the figure was 32%.

If we now use these new figures for the area occupied by follicle cells, the ratio $A_{10}/A_5 = 2.06$. This is closer to the observed value for h_5/h_{10} of 1.7, and suggests that much of the compensation for the increase in the surface area of the oocyte takes place by an increase in intercellular space. The remaining discrepancy suggests an increase in volume of the cells during mid vitellogenesis. This increase can be calculated from the above figures, and Table 4 sets out the results for such calculations in normal and allatectomized females.

The above data confirm once more that in the normal mated female there is a decrease in the volume of the follicle cells as the result of JH action. Thus, the volumes of the follicular layer are always markedly less in the normal females, when compared with those from allatectomized females. The abrupt decrease in volume which occurs *in vitro* as the result of exposure to JH, and which might be expected between intervals 4 and 5 when patency first appears, is almost completely masked *in vivo* by a marked increase in volume of the cells. Thus, in allatectomized females, the volume of the follicular epithelium increases by a factor of 1.6 between intervals 4 and 5. Assuming that the same sort of increase occurs over the same growth period in follicles from normal females, the reduction in volume due to JH action in normal females is such that the volume of the cells at interval 5 is approximately 60% of that at interval 4.

This is a somewhat smaller decrease in volume than that obtained by other methods, but the present calculations do not take into account the fact that the follicle cells at the anterior and posterior ends of the follicle fail to exhibit patency at any stage.

The clear increase in volume of the cells in both normal and allatectomized females during mid to late vitellogenesis is probably a consequence of the synthesis of secondary coat precursors by the follicle cells. This activity will mask the response of the follicle cells to JH, and compensate in part at least for the rapid increase in surface area of the follicle attendant upon the growth of the oocyte.

DISCUSSION AND CONCLUSIONS

JH has been shown previously to act on the follicle cells of the vitellogenic oocytes of *Rhodnius* so that spaces appear between the cells (Pratt & Davey, 1972; Davey & Huebner, 1974). This alteration in morphology might be a consequence of two sorts of response. On the one hand, hypothetical contractile elements associated with the cytoskeleton of the cell might act to bring about an increase in height of the cell, thus reducing its diameter, and causing spaces to appear between the cells. On the other hand, the volume of the cell might be reduced by pumping out fluid. This would also bring about a decrease in diameter of the cell, providing that its height, as a consequence of cytoskeletal elements, did not decrease proportionately. Since both of these hypotheses require an intact cytoskeletal system, it is no surprise that disrupters of cytoskeletal integrity interfere with the development of patency as a result of JH action (Abu-Hakima, 1976).

In the present work, direct measurements of the dimensions of follicle cells from fixed ovarioles and determinations of the fluid content by interference microscopy both demonstrate that the cells respond to JH by shrinking to about 50% of their original volume. This does not occur in the cells from allatectomized females upon exposure to JH. This is direct support for the second hypothesis. Moreover, the follicle cells do not increase, but decrease, in height, and cells from allatectomized females fail to undergo this same decrease at activation. This is direct evidence against the first hypothesis. The fact that the cells from allatectomized females fail to respond to JH by reducing their volume confirms an earlier observation that the follicle cells from allatectomized females fail to respond to JH as measured in the patency test (Abu-Hakima & Davey, 1975).

We are therefore persuaded that JH acts on the follicle cells in at least two quite different ways. JH is first necessary during the previtellogenic phase of growth to allow the follicle cells to become competent to respond to the gonadotropin at a later stage. We can offer little concerning the nature of the differentiative step except to remark that it recalls the concept of 'prerequisite hormone priming', as exemplified in the induction of vitellogenin synthesis in amphibian liver (Wallace & Berjink, 1974; Wallace & Jared, 1969; Whittliff & Kenney, 1972).

The second response to JH requires this priming, and consists of a pumping out of fluid from the cell, resulting in a decrease in volume. While the data presented here do not allow us to explore a more precise description of the action of JH, they suggest that the primary target of the hormone might be the membrane, as has been suggested by Wigglesworth (1957, 1969) and Baumann (1968, 1969). This possibility is currently under investigation.

The response to JH is not the only factor which governs the volume of the follicle cells. As the oocyte grows, its surface area increases dramatically and the follicular epithelium is subjected to stress which would tend to increase the size of the inter-follicular cell spaces and/or decrease the height of the epithelium. While an increase in the size of the spaces helps to compensate for the increased surface area, there is also a dramatic increase in volume, presumably a result of the synthesis of secondary coat precursor.

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REFERENCES

- ABU-HAKIMA, R. (1976). The action of juvenile hormone on follicle cells in the insect *Rhodnius prolixus* Stål. Ph.D. thesis, McGill University, Montreal.
- ABU-HAKIMA, R. & DAVEY, K. G. (1975). Two actions of JH on follicle cells of *Rhodnius prolixus* Stål. *Can. J. Zool.* **53**, 1187-8.
- BARER, R. & JOSEPH, S. (1955a). Refractometry of living cells. II. The immersion medium. *Q. Jl microsc. Sci.* **96**, 1-27.
- BARER, R. & JOSEPH, S. (1955b). Refractometry of living cells. III. Technical and optical methods. *Q. Jl microsc. Sci.* **96**, 423-447.
- BAUMANN, G. (1968). Zur Wirkung des Juvenilhormons: Elektrophysiologische Messungen an der Zellmembran der Spiecheldrüse von *Galleria mellonella*. *J. Insect Physiol.* **14**, 1459-76.
- BAUMANN, G. (1969). Juvenile hormone: effects on bimolecular lipid membranes. *Nature, Lond.* **223**, 316-17.
- DAVEY, K. G. & HUEBNER, E. (1974). The response of the follicle cells of *Rhodnius prolixus* to juvenile hormone and antigonadotropin *in vitro*. *Can. J. Zool.* **52**, 1407-12.
- DAVIES, H. G. (1958). The determination of mass and concentration by microscope interferometry. In *General Cytochemical Methods* (ed. J. F. Danielli), pp. 57-151. New York: Academic Press.
- PRATT, G. E. & DAVEY, K. G. (1972). The corpus allatum and oogenesis in *Rhodnius prolixus* (Stal). I. The effects of allatectomy. *J. exp. Biol.* **56**, 201-14.
- WALLACE, R. A. & BERGINK, E. W. (1974). Amphibian vitellogenin properties, hormonal regulation of hepatic synthesis and ovarian uptake, and conversion to yolk proteins. *Am. Zool.* **14**, 1159-75.
- WALLACE, R. A. & JARED, D. W. (1969). Studies on amphibian yolk. VIII. The estrogen-induced hepatic synthesis of a serum lipophosphoprotein and its selective uptake by the ovary and transformation into yolk platelet proteins in *Xenopus laevis*. *Dev. Biol.* **19**, 498-526.
- WHITTLIFF, J. L. & KENNEY, J. F. (1972). Regulation of yolk protein synthesis in amphibian liver. I. Induction of lipovitellin synthesis by estrogen. *Biochim. biophys. Acta* **269**, 485-92.
- WIGGLESWORTH, V. B. (1957). The action of growth hormones in insects. *Symp. Soc. exp. Biol.* **11**, 204-27.
- WIGGLESWORTH, V. B. (1969). Chemical structure and juvenile hormone activity: comparative tests on *Rhodnius prolixus*. *J. Insect Physiol.* **15**, 73-94.