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THE ACTION OF MOULTING HORMONE AND JUVENILE HORMONE AT THE CELLULAR LEVEL IN *RHODNIUS PROLIXUS*

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In the course of a discussion on the mode of action of growth hormones in insects (Wigglesworth, 1957*a*) a preliminary account was given of the cytological changes brought about by the moulting hormone in certain selected tissues. Further work, with the use of improved methods, has substantiated many of these earlier results; others require revision.

Growth and moulting in *Rhodnius* are initiated by the meal of blood, which may amount to ten times the initial weight of the insect; and the chief difficulty in defining the action of the moulting hormone is that of differentiating between (i) the effects of distension of the abdomen, (ii) the effects of nutrition, and (iii) the true effects of the hormone itself.

In the earlier work the method adopted for distinguishing between (ii) and (iii) was to decapitate the 4th-stage larva immediately after feeding and then to compare untreated decapitated larvae with similar larvae that had been injected with the purified moulting hormone ecdyson.

In these experiments the first visible changes, enlargement of the nucleolus, increase in ribonucleoprotein (RNA) in the cytoplasm around the nuclei, and swelling of the mitochondria, were detectable within 6 hr. after injection of the hormone. By the end of 24 hr. the nucleolus had enlarged considerably, cytoplasmic RNA had increased strikingly and mitochondria were both swollen and multiplying.

These changes were observed in the epidermal cells of the abdominal wall, in the fat-body cells and in the sternal intersegmental muscles. It was concluded that the essential action of the hormone was to restore the capacity of the cells for protein synthesis and thus for growth. It was noted, however, that decapitation appeared not to interfere with these changes in the cells of the fat body. It was suggested that perhaps the fat body had a lower threshold for the action of the moulting hormone and reacted to small amounts of the hormone liberated during the act of feeding. In all three tissues cell division does not occur until these growth changes are well advanced.

METHODS

These earlier experiments suffered from two major defects. First, they did not take into account the effects of distension. Secondly, it may be that the nutrition of the tissues is impaired by decapitation; for the loss of the neurosecretory system in the

brain has been shown by Thomsen & Møller (1959) to have a profound effect on the secretion of protease in the mid-gut of *Calliphora*.

These defects have been got over by using decapitated 4th-stage larvae joined in parabiosis to suitable partners.

(i) The effects of nutrition alone, uncomplicated by distension or by the presence of moulting hormone, were observed by joining decapitated unfed 4th-stage larvae with recently fed adults.

(ii) The effects of nutrition, without distension, but combined with the moulting hormone, were observed in unfed 4th-stage larvae joined to 4th-stage larvae with the tip of the head removed after the critical period (4 days after feeding).

(iii) The effects of distension were inferred by comparing the changes in fed and unfed larvae treated as in (ii).

(iv) The effects of the juvenile hormone were inferred by comparing decapitated 4th-stage larvae, fed and unfed, when joined (*a*) to 4th-stage larvae which retained the corpus allatum and (*b*) to 5th-stage larvae which likewise retained the corpus allatum.

The cells and tissues were studied in whole mounts of the dorsal and ventral walls of the abdomen, attention being concentrated on the epidermal cells, fat body and sternal intersegmental muscles.

For lipid-containing structures (nucleoli, mitochondria, fat reserves) the isolated tergites and sternites were fixed for 1 hr. in 1 % osmium tetroxide in Ringer's solution, washed for 5 min., and stained by immersion in a saturated solution of ethyl gallate for 24 hr. or longer (Wigglesworth, 1957*b*). Storage for a week or 10 days in the ethyl gallate is useful to dissolve away the red pigment in the epidermal cells. They were then passed through 50 % alcohol, equal parts of 75 % alcohol and cellosolve, pure cellosolve (two changes), equal parts cellosolveand thin cedar oil, pure thin cedar oil containing ethyl gallate, and then mounted in thick cedar oil saturated with ethyl gallate.

For nuclear changes (without regard to other cell structures) the tissues were fixed in Carnoy's solution and stained with Hansen's trioxyhaematein. For the demonstration of chromosomes in the presence of mitochondria, nucleoli, etc., the isolated abdominal wall was pretreated with acid (0·002 M acetic acid in Ringer's solution for 3 min. or saturated carbonic acid in Ringer's solution for 1 min.) before fixation with osmium tetroxide and staining with ethyl gallate (Wigglesworth, 1963).

For nucleic acids and glycogen the tergites and sternites were fixed in Carnoy's solution, stained by the periodic acid-Schiff (PAS) method (with diastase-treated controls) and then with 0·025 % toluidine blue for 1 hr. at room temperature (with ribonuclease-treated controls), passed directly through several changes of absolute alcohol, cleared in xylol and mounted in 'crystallite'. Confirmatory results were obtained with methyl green and pyronin staining.

CHANGES IN THE CELLS DURING FASTING

In order to demonstrate the effects of nutrition and of the moulting hormone more clearly, the insects were kept at 26° C. without feeding until their reserves were nearly exhausted. The changes that take place in the 4th-stage larva during fasting, in the tissues selected for study, are as follows.

(i) *Epidermal cells*

At the time of moulting, when the cuticle is still being secreted, the cytoplasm of the epidermal cells is filled with filamentous mitochondria and abundant RNA. The cytoplasm is rich in endoplasmic reticulum which gives a diffuse staining with osmium-ethyl gallate (Wigglesworth, 1957*a*, 1961). The nucleolus is conspicuous.

During the weeks of starvation that follow, the nucleolus (as seen in osmium-ethyl gallate preparations) becomes progressively smaller, but it remains a distinct and uniform rounded body after 16 weeks' starvation. On the other hand, the nucleolus as revealed by toluidine blue staining not only becomes smaller but the RNA content disappears completely, so that by 12 weeks the position of the nucleolus is recognizable only as a rounded unstained space marked out by granules of desoxyribonucleic acid (DNA) applied to its surface.

In the first 2 weeks of starvation, rounded or oval deposits appear in the cytoplasm of the epidermal cells; they often have an osmiophil cortex and a pale centre, and they also stain with toluidine blue. Gradually they disappear and cytoplasmic RNA becomes barely detectable. The mitochondria become sparse, the cytoplasm between the mitochondria being unstained by toluidine blue or osmium-ethyl gallate, although it may contain a few brown osmiophil spheres.

It is suggested that the RNA-containing osmiophil deposits appearing early during fasting are breakdown products of the endoplasmic reticulum; and that the osmiophil spheres appearing in the later stages of starvation are breakdown products of the mitochondria.

(ii) *Sternal muscles*

As was described in an earlier paper (Wigglesworth, 1956) the myofibrils of the sternal intersegmental muscles undergo rapid autolysis during the first few days after moulting. The nuclei, with small nucleoli, persist within the muscle sheath together with a minimal amount of cytoplasm containing a few mitochondria. The cytoplasmic RNA quickly disappears and in extreme starvation the nucleoli lose their RNA and acquire the same appearance as in the epidermal cells.

(iii) *Fat body*

At the time of moulting the fat-body cells are filled with fat droplets. The thin strands of cytoplasm between the droplets and around the nucleus contain deposits of glycogen, a small amount of RNA, and abundant filamentous mitochondria which are obscured in whole mounts by the great quantities of stored fat.

During starvation fat and glycogen are steadily reduced. Some glycogen may still be detectable after 12 weeks. With the reduction in the amount of fat the filamentous mitochondria become apparent (Fig. 1 A). In advanced starvation, exceeding 16 weeks, the droplets of neutral fat, which stain blue-black with osmium-ethyl gallate in the well-nourished insect, stain a pale blue. This was attributed to the preferential utilization of the unsaturated lipids leading to a fall in the osmium-binding capacity of the fats (Wigglesworth, 1957*b*).

As starvation continues the mitochondria become fewer and among them there appear rounded deposits which stain a deep brown with osmium-ethyl gallate (Fig. 1 B).

These become more and more conspicuous as the mitochondria disappear; and in the final stages of starvation (20 weeks or later) the shrunken rounded fat-body cells contain abundant droplets of this type and only a few small mitochondrial granules (Fig. 1 C). It seems likely that these inclusions represent the residue of 'dead' and disintegrated mitochondria. They differ from droplets of neutral fat in staining brownish black (and not blue-black) with osmium-ethyl gallate and in failing to take up Sudan III; and they differ from mitochondria in staining more deeply by the osmium procedure (although there are occasional small granules which are not distinguishable with certainty from mitochondria), in staining with Hansen's haematoxylin, and in darkening after osmium fixation alone without treatment with ethyl gallate.

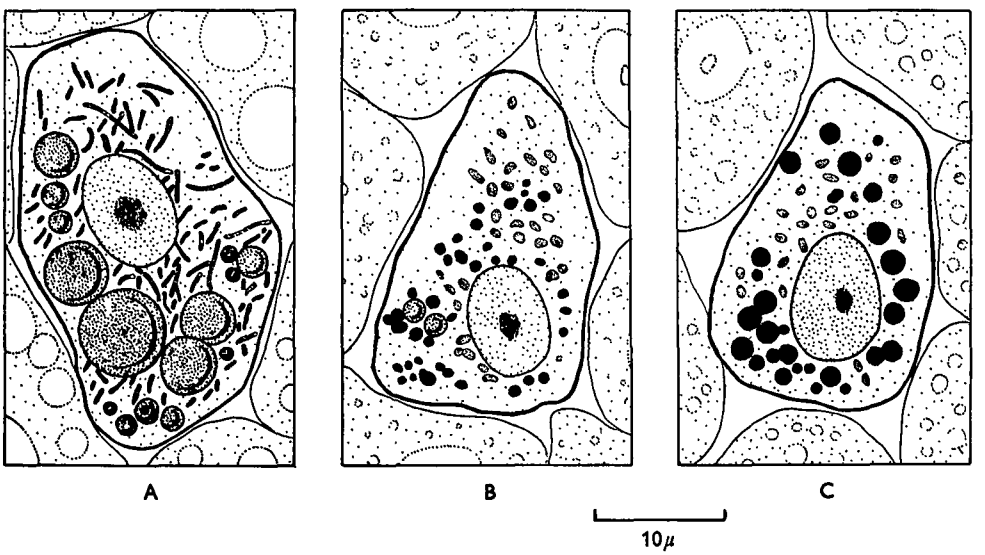

Fig. 1. Effect of starvation on fat-body cells of 4th-stage larva starved at 26° C.: osmium-ethyl gallate staining. A, starved 12 weeks; fat droplets and mitochondria. B, 20 weeks; two fat droplets, pale mitochondria and brownish black osmiophil inclusions. C, 20 weeks, extreme starvation; no fat droplets, a few pale mitochondria, large osmiophil inclusions.

In the late stages of starvation RNA has almost disappeared from the cytoplasm of the fat-body cells; the nucleolus is small and evenly rounded as seen after staining with osmium-ethyl gallate (Fig. 1 C), unstained but covered with granules of DNA after staining with toluidine blue (Fig. 5 A).

EFFECTS OF NUTRITION AND MOULTING HORMONE

4th-stage larvae starved at 26° C. for 16–20 weeks were used for these experiments. To show the effects of nutrition alone they were decapitated and joined in parabiosis to the tip of the head of adult *Rhodnius* fed 1 day earlier. To show the effects of nutrition plus moulting hormone they were joined in parabiosis to 4th-stage larvae with the head cut through at 4 days after feeding.

The tissues were examined in groups of six individuals for each treatment: (*a*) at the outset; (*b*) at 1 day after parabiosis; (*c*) at 4 days after parabiosis. The tergites of the abdomen were treated by the osmium-ethyl gallate method; the sternites of the same insect were stained with PAS and toluidine blue after fixation with Carnoy's solution.

(i) *Epidermal cells*

The results on the epidermal cells are summarized in Fig. 2. In the fasting insect stained with osmium-ethyl gallate (Fig. 2A) the nucleoli are small; the cytoplasm contains only a few slender mitochondrial filaments and minute granules.

When exposed to 'nutrition' alone for one day (Fig. 2B) there is no significant change. After 4 days (Fig. 2C) there is again no constant difference from the condition in the fully starved insect.

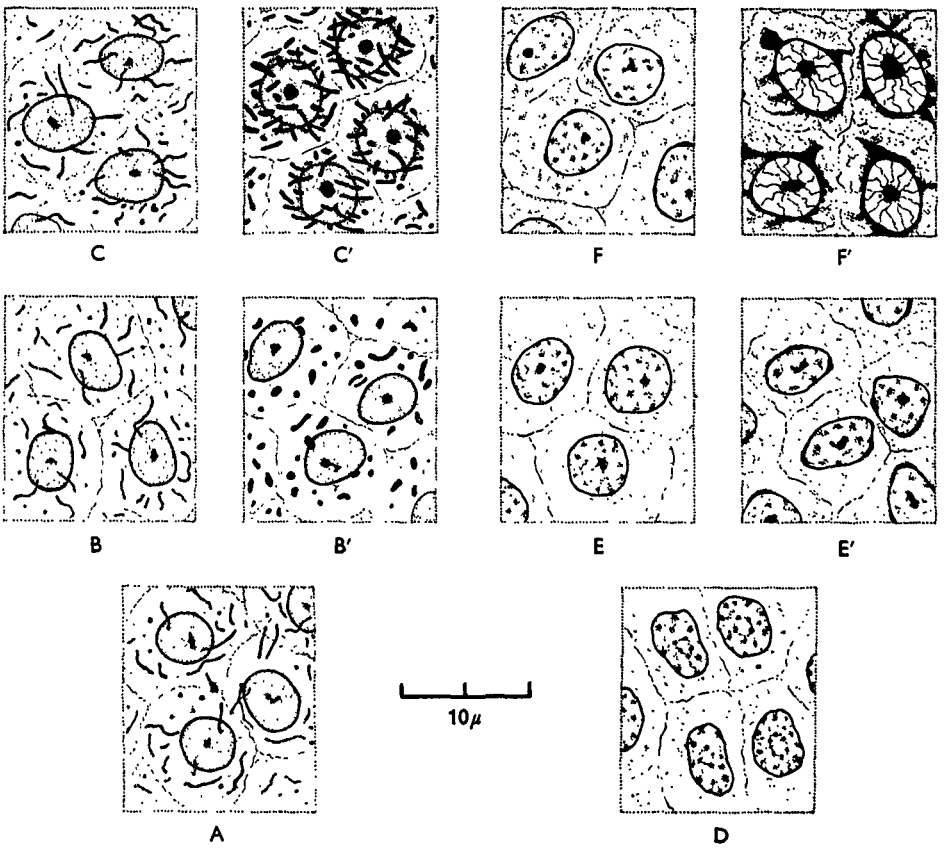

Fig. 2. Effect of 'nutrition' and moulting hormone on epidermal cells of 4th-stage larva; A–C, osmium-ethyl gallate showing mitochondria; D–F, Carnoy-toluidine blue showing nucleic acids. A and D, fully starved condition. B and E, exposed to 'nutrition' alone for 1 day. B′ and E′, 'nutrition' plus moulting hormone for 1 day. C and F, 'nutrition' alone for 4 days. C′ and F′, 'nutrition' plus moulting hormone for 4 days.

When exposed to 'nutrition' plus moulting hormone for one day (Fig. 2B′) the nucleoli are distinctly enlarged and the mitochondria are swollen. After 4 days (Fig. 2C′) the nuclei are slightly enlarged, the nucleoli strikingly so; there is a diffusely staining zone in the cytoplasm around the nucleus, and the mitochondria which are increasing in numbers are in the form of thickened rods and granules.

In the fasting insect fixed with Carnoy and stained with toluidine blue (Fig. 2D) the nuclei tend to be somewhat shrunken and irregular in outline. The nucleoli are

unstained but demarcated by granules of DNA applied to the surface. There is almost no detectable RNA in the cytoplasm.

When exposed to 'nutrition' alone for one day (Fig. 2E) the nuclei are evenly rounded and RNA has appeared in the nucleolus; there is no detectable increase in the RNA in the cytoplasm. After 4 days (Fig. 2F) minute amounts of RNA are detectable as slender crescents applied to the nuclear membrane, but there is no other change.

When exposed to 'nutrition' plus moulting hormone for one day (Fig. 2E') there is a greater accumulation of RNA in the nucleolus than in the control, and conspicuous crescents of RNA are appearing on the nuclear membrane.

After 4 days (Fig. 2F') the nuclei are enlarged; the nucleoli are greatly enlarged with chromatin filaments running from them to the nuclear membrane. There are large deposits of RNA in the cytoplasm, particularly around the nucleus.

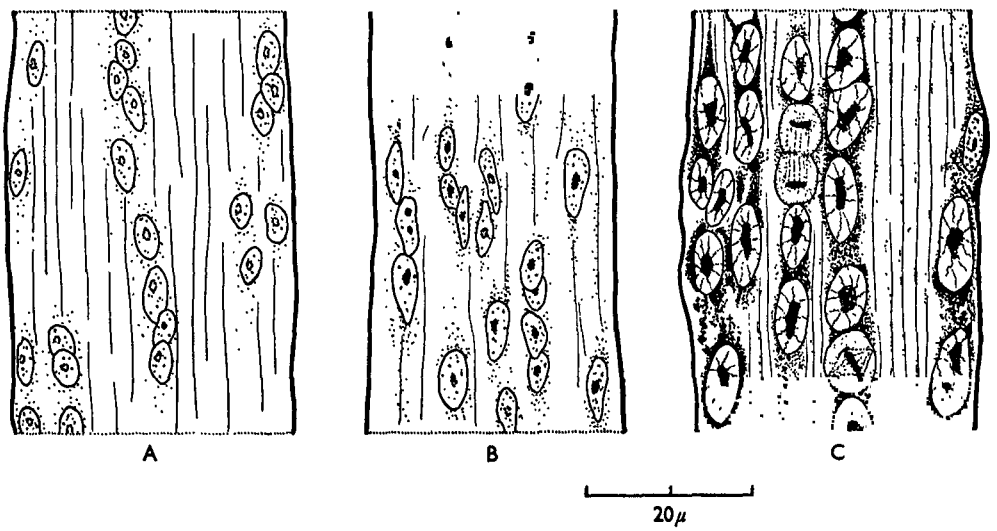

Fig. 3. Effect of 'nutrition' and moulting hormone on ventral abdominal muscles of 4th-stage larva: Carnoy-toluidine blue. A, fully starved condition. B, exposed to 'nutrition' alone for 4 days. C, exposed to 'nutrition' plus moulting hormone for 4 days.

(ii) *Sternal intersegmental muscles*

Preparations stained with osmium and ethyl gallate confirm the observations recorded in the earlier paper (Wigglesworth, 1957*a*). There is almost no change in the muscles exposed to nutrition alone. When the moulting hormone is present in addition, enlargement of the nucleolus is just detectable after 1 day. After 4 days the nuclei and nucleoli are greatly enlarged and mitochondria are becoming far more numerous in the cytoplasm.

Fig. 3 summarizes the results obtained with toluidine blue staining. In the fully starved insect (Fig. 3A) the nucleoli are small; RNA is barely detectable in the cytoplasm around the nuclei. There is a very slight increase in the size of the nucleoli and in cytoplasmic RNA after 1 day in the insects exposed to 'nutrition' plus moulting hormone. After 4 days with 'nutrition' alone (Fig. 3B) the nucleoli are still small and rather pale, and there is a very slight increase in the RNA in the cytoplasm. After

4 days with 'nutrition' plus moulting hormone (Fig. 3C) the nuclei are swollen, with greatly enlarged nucleoli and radiating chromatin filaments; mitoses are occurring; there are large amounts of RNA between the rows of nuclei; and muscle fibrils are forming.

(iii) *Fat body*

Whereas 'nutrition' alone has almost no effect on the histological appearance of the epidermal cells and the sternal intersegmental muscles, 'nutrition' plus moulting hormone quickly restores the cellular activities of ribonucleoprotein synthesis and mitochondrial increase that are needed for growth. But in the cells of the fat body there is no detectable difference between the effects of the two treatments.

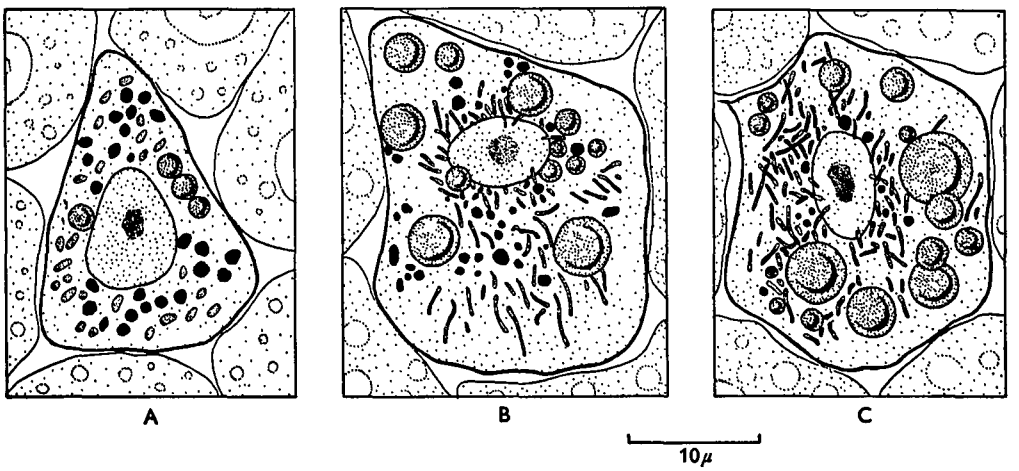

Fig. 4. The identical effect of 'nutrition' alone and 'nutrition' plus moulting hormone on the fat-body cells: osmium-ethyl gallate. A, starved for 20 weeks (cf. Fig. 1B). B, 'nutrition' alone for 1 day; fat droplets and mitochondria increasing in number, osmiophil inclusions diminishing. C, 'nutrition' plus moulting hormone for 4 days; further increase in fat droplets and mitochondria, dark brown osmiophil inclusions disappearing.

The identical changes that take place in the fat-body cells of starved insects in parabiosis with either fed adults or fed 4th-stage larvae, as seen after osmium-ethyl gallate staining, are summarized in Fig. 4. The condition in the insect starved for 20 weeks (Fig. 4A) has already been described (cf. Fig. 1 B and p. 234). After one day (Fig. 4B) the nucleolus is enlarged, the brown inclusions believed to be products of mitochondrial breakdown (p. 234) are still visible, but fresh droplets of neutral fat are appearing. Mitochondrial filaments are now present at the periphery of the cells, while abundant new mitochondria are appearing, particularly in the neighbourhood of the nucleus. After 4 days (Fig. 4C) the mitochondria have increased still further, droplets of neutral fat are more plentiful, the dark brown inclusions are becoming less numerous.

The changes in nucleic acid and glycogen are summarized in Fig. 5. In the fully starved condition, at the end of 20 weeks, glycogen is absent (Fig. 5A), the body of the nucleolus shows no staining for RNA (cf. p. 233) and there is very little RNA in the cytoplasm (Fig. 5A'). At one day after parabiosis plenty of glycogen is appearing between the fat vacuoles (Fig. 5B) and RNA is present in the nucleolus and in the

cytoplasm, both around the nucleus and the periphery of the cells (Fig. 5 B'). At 4 days after parabiosis (Fig. 5 C) glycogen continues to accumulate. The RNA in the nucleolus is still more conspicuous and much more RNA is present in the cytoplasm between the fatty and aqueous vacuoles (Fig. 5 C').

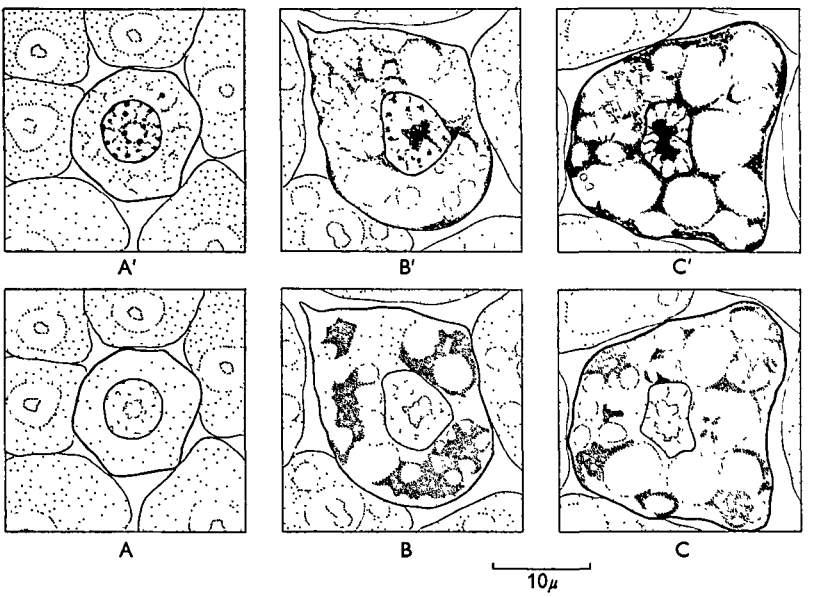

Fig. 5. The identical effect of 'nutrition' alone and 'nutrition' plus moulting hormone on the fat-body cells: Carnoy-PAS-toluidine blue. A, starved 20 weeks, showing absence of glycogen. A', the same cell showing absence of RNA in the nucleolus and very little RNA in the cytoplasm. B, 'nutrition' alone for 1 day, showing glycogen in the cytoplasm. B', the same cell showing RNA in nucleolus and cytoplasm. C, 'nutrition' plus moulting hormone for 4 days, showing glycogen. C', the same cell showing nucleic acids.

INDUCTION OF MITOSIS

(i) *Epidermis*

It was shown in earlier work that the moulting hormone does not of itself initiate mitosis in the epidermis. It merely induces 'activation' of the cells. This 'activation', characterized by the enlarged nucleolus, increase in mitochondria and appearance of RNA in the cytoplasm, was later described as evidence of renewed protein synthesis (Wigglesworth, 1957*a*) which leads on to the complex sequence of changes that result in the deposition of the new cuticle. Mitosis is not a necessary step in this moulting process.

Mitosis is brought about by the mutual separation of the activated cells; and that applies both to the activation induced by the moulting hormone (Wigglesworth, 1940) and to the identical activation induced by local injury (Wigglesworth, 1937). In both these circumstances mitosis occurs only when the nuclei of neighbouring epidermal cells are unduly separated from one another: after the stretching caused by feeding in the growing insect, or in the sparse zone around a wound produced by the centripetal migration of epidermal cells.

These results have been confirmed during the present experiments. 4th-stage

larvae decapitated at one day after feeding and joined in parabiosis with 4th-stage larvae with the corpus allatum intact (Fig. 6A) show abundant mitoses in the epidermis by 4 days after feeding. Over the measured area of cuticle used for the drawings in Fig. 6A the number of nuclei increased from 28 to 59. Unfed 4th-stage larvae similarly treated (Fig. 6B) show very few mitoses, which may indeed be difficult to find. In the measured areas illustrated the number of nuclei happened to remain unchanged at about 58.

The cytological changes in the nucleus and nucleolus during mitosis in the epidermis of *Rhodnius* will be described elsewhere (Wigglesworth, 1963). It will suffice to report here that already before the spireme stage of prophase the nucleolus separates into two

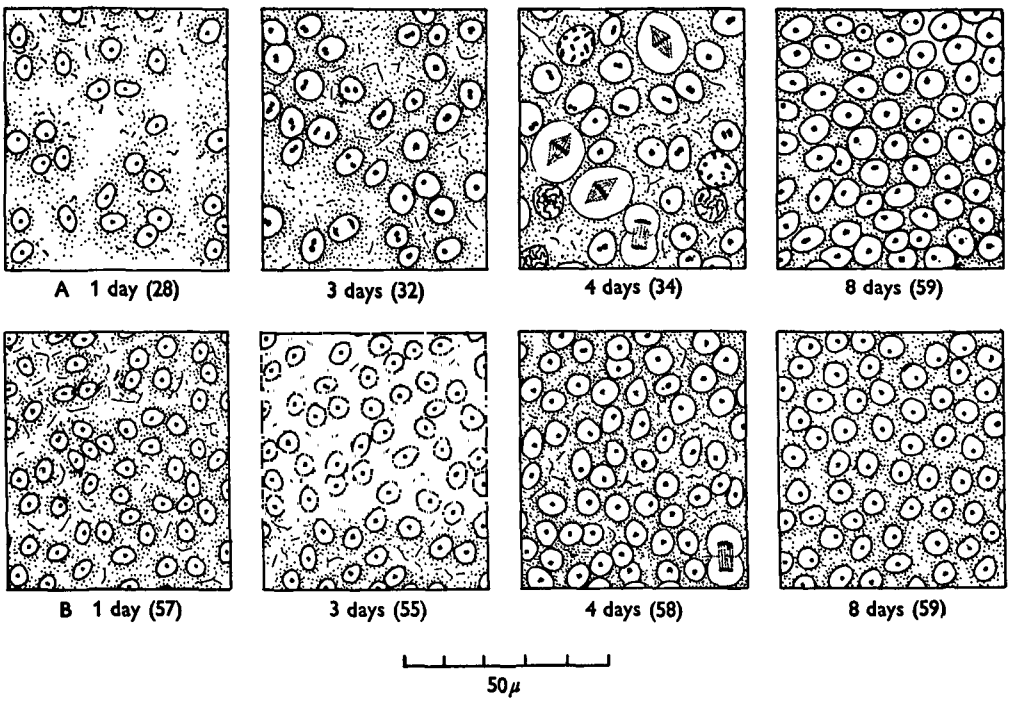

Fig. 6. A, epidermis of 4th-stage larva stretched by feeding and exposed to moulting hormone (plus juvenile hormone). B, epidermis of unstretched larva likewise exposed to moulting hormone (plus juvenile hormone). The number of days after the beginning of the experiment is indicated; the figures in brackets show the number of nuclei present in the measured area selected for drawing.

halves which move apart in a manner reminiscent of a diminutive 'mitosis' inside the nucleus. This change is already evident in the epidermis of the stretched insect within 3 days of exposure to the moulting hormone (Fig. 6A); it is almost absent in the unstretched epidermis (Fig. 6B).

It is not surprising that a few mitoses do occur in the unfed 4th-stage larva when it is induced to moult, because, at the time of ecdysis in the 3rd-stage larva, there is some increase in the surface area of the abdominal wall as the result of the swallowing of air by the newly moulted insect. To this small extent even the unfed 4th-stage larvae have been exposed to some degree of stretching. It is noteworthy that the nuclei are consistently smaller in the unfed insect.

(ii) *Sternal intersegmental muscles*

The small degree of stretching that follows ecdysis will affect also the ventral abdominal muscles. The nuclei in these muscles show some mitoses when the unfed insect is induced to moult (Fig. 3C), but these are far fewer than in the fully stretched insect after feeding.

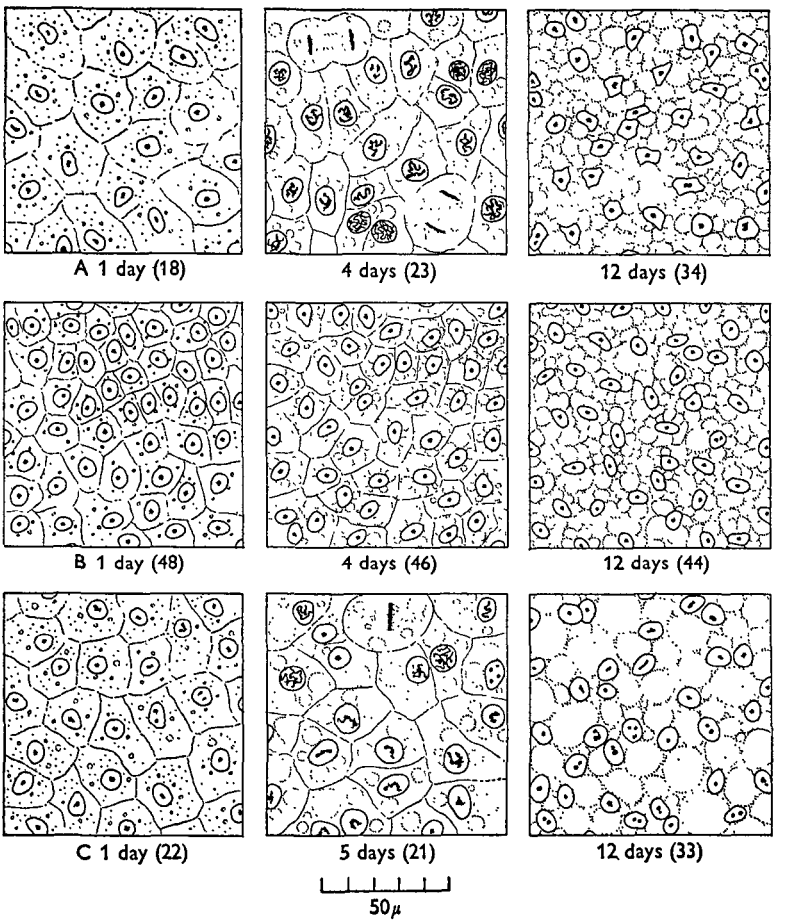

Fig. 7. A, the single layer of fat-body cells below the abdominal tergites of 4th-stage larva stretched by feeding and exposed to moulting hormone. B, the same in unstretched larva exposed to 'nutrition' plus moulting hormone. C, the same in larva decapitated after feeding (exposed to 'nutrition' in absence of moulting hormone). The number of days after the beginning of the experiment is indicated; the figures in brackets show the number of nuclei present in the measured area selected for drawing.

(iii) *Fat body*

Since the nuclei of the fat body, unlike those of the epidermis and ventral muscles, become 'activated' by 'nutrition' alone, in the absence of the moulting hormone, it was of interest to see whether, under these conditions, they would go on to mitosis.

Fig. 7A shows the nuclear changes taking place in the single layer of fat-body cells of the fully fed 4th-stage larva moulting in the normal way. By 4 days after feeding the

nucleoli had become exceedingly convoluted, many nuclei were in the spireme stage, and in others mitosis was in progress. By 12 days after feeding the number of nuclei per unit area had almost doubled.

In the unfed insect exposed to the moulting hormone by joining in parabiosis with a moulting 4th-stage larva (Fig. 7B) the nucleoli increased in size but did not become convoluted; mitoses failed to occur (or were very few) and there was no increase in the number of nuclei per unit area.

Finally, in the 4th-stage larva stretched by feeding in the normal way, but deprived of the moulting hormone by decapitation after feeding, the sequence of changes was similar to that in the moulting insect. Mitoses appeared in the 'activated' cells within 5 days after feeding (Fig. 7C), and by 12 days the number of nuclei per unit area showed a 50% increase. In these same insects there was, of course, no sign of activation or moulting in the epidermis or ventral muscles.

EFFECT OF JUVENILE HORMONE

Once the process of growth and moulting has been set going by the moulting hormone, the presence or absence of the juvenile hormone determines whether the cuticular structures laid down by the epidermis are to be of larval or adult type.

It has not been possible with the methods employed to detect any constant differences in the cytological characters of activated epidermal cells with and without the juvenile hormone. Differences in cuticle pigmentation, in the degree of sclerotization, and in the sculpturing of the cuticle surface, etc., show that there must be differences in the character and distribution of the enzymes in the epidermal cells; but these are not associated with any differences visible in histological preparations.

The detectable differences are those dependent on the mutual relations between cells. In the general epidermis of the abdomen the number of cells per unit area below the newly formed cuticle is much greater in the larva than in the adult: 223 per 10,000 μ^2 in the unfed 5th-stage larva, 93 per 10,000 μ^2 in the adult (Wigglesworth, 1942). This difference becomes evident when a comparison is made between decapitated 4th-stage larvae caused to moult by joining in parabiosis to 4th-stage larvae with the corpus allatum intact (Fig. 6A) and similar larvae joined in parabiosis to moulting 5th-stage larvae (Fig. 8A), that is, in the absence of the juvenile hormone. At 3 days after feeding divided nucleoli are much less numerous in the series without the juvenile hormone (Fig. 8A), at 4 days there are fewer mitoses, and in the completed epidermis at 12 days the number of nuclei per unit area shows an increase of only about 50% (as compared with more than 100% in the series provided with juvenile hormone (Fig. 6A)).

As was seen in Fig. 6B, when the unfed 4th-stage larva is caused to moult by joining to a moulting 4th-stage larva with corpus allatum intact, the numbers of mitoses are far fewer than in the insect stretched by feeding (Fig. 6A). But when the unfed 4th-stage larva is induced to moult by joining to a moulting 5th-stage larva, that is, in the absence of the juvenile hormone, there are already many more nuclei in the epidermis than are required for the adult type of cuticle. Not only does mitosis appear to be completely absent, but the excess nuclei are eliminated; by 6 days after joining many pycnotic and degenerating nuclei giving rise to 'chromatic droplets'

are to be seen among the epidermal cells (Fig. 8B), and by 12 days when the new cuticle is being laid down the number of nuclei per unit area has been reduced by about one-third of what it was at the outset.

In regions where extra growth occurs at metamorphosis, as at the sides of the abdomen where the lateral pleats are developed in the adult, or in the wing lobes which grow enormously to form the adult wings, the effects are naturally reversed, and it is in the absence of the juvenile hormone that excessive cell division takes place.

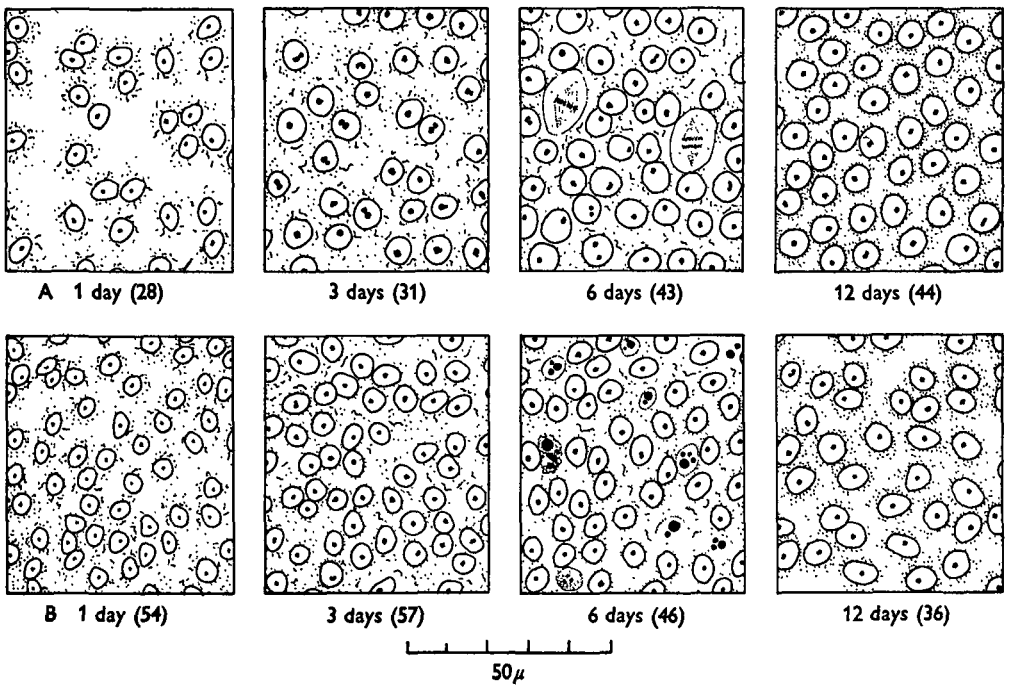

Fig. 8. A, epidermis of 4th-stage larva stretched by feeding and exposed to moulting hormone (in absence of juvenile hormone). B, epidermis of unstretched larva likewise exposed to moulting hormone without juvenile hormone. The number of days after the beginning of the experiment is indicated; the figures in brackets show the number of nuclei present in the measured area selected for drawing.

DISCUSSION

(i) *Action of the moulting hormone*

In seeking to define the mode of action of the moulting hormone, the aim in the past has been to find some process in the metabolism of the cell for which the hormone provides a necessary ingredient.

It was pointed out that the unfed larva of *Rhodnius*, or the larva decapitated after feeding, is in a state of suppressed growth and depressed metabolism equivalent to diapause; and when this state was shown to be due to the lack of a moulting hormone it was suggested that natural diapause in other insects would probably prove to be due to the same cause (Wigglesworth, 1934). That was confirmed experimentally by Williams (1946) in the diapausing pupa of *Hyalophora cecropia*; and since the diapause state was associated with a great reduction in the activity of the cytochrome system,

notably an extremely low content of cytochrome C, Williams (1951) suggested that the synthesis of cytochrome C was the key deficiency that was made good by the moulting hormone. But there were certain tissues (heart, abdominal muscles) in the diapausing cecropia pupa which had a normal cytochrome system; in *Rhodnius* and other insects the system was fully active in much of the body during diapause; finally, Shappirio & Williams (1957) themselves showed that there was no absolute lack of cytochrome C in the diapausing cecropia but only a general reduction in the cytochrome system with a relatively great preponderance of cytochrome oxidase. Synthesis of the component enzymes of the cytochrome system was just one of the consequences of renewed growth.

It was later suggested that the key deficiency in the diapausing *Rhodnius* was a failure in protein synthesis and that the primary effect of the moulting hormone was to restore the capacity for protein synthesis by the cells and thus to renew their capacity for growth. The first cytological effects of the moulting hormone to be described were the enlargement of the nucleolus and the increased staining of the cytoplasm. These changes, involving ribonucleoprotein formation with greatly increased mitochondrial activity, were recognized as the usual cytological picture associated with active protein synthesis. The further hypothesis was suggested that the moulting hormone might bring about this change by affecting permeability relations within the cells and so allowing access of enzymes to their substrates.

This interpretation that 'activation' (that is, renewed protein synthesis) is the essential effect of the moulting hormone, suffers from precisely the same defects as the cytochrome C hypothesis of Williams: it lacks universality. As shown in the foregoing experiments it is applicable in *Rhodnius* to the epidermal cells and to the ventral abdominal muscles; but it does not apply to the cells of the fat body. When these are provided with the normal nutrients in the blood, in the absence of the moulting hormone, they show the same cytological changes as the activated epidermal cells.

One must conclude that the moulting hormone is not a necessary ingredient for the growth of insect cells in general. But that the epidermis (and certain other tissues) are so constituted that they are normally dormant and become 'activated' and grow only when they are exposed to the hormone. The hormone acts as a 'messenger' which provokes the growth response. This provision is perhaps needed in order to ensure the cyclical character of insect growth, which is necessitated by the existence of a cuticle that must be cast and renewed at defined periods. Precisely the same response is provoked by 'wound hormones' from injured tissue (Wigglesworth, 1937); that is obviously necessary to ensure repair.

The first visible effect of this re-activation of the epidermal cell is in the nucleolus. The nucleolus is a chromosomal organelle. In the salivary gland nuclei of *Drosophila* and *Chironomus* the place of the nucleolus is partly taken by the Balbiani rings and 'puffs' on the chromosomes themselves. It is therefore of great interest that the earliest visible effect of the moulting hormone (ecdyson) in *Chironomus* is the formation of certain specific 'puffs' on the giant chromosomes (Clever, 1961).

(ii) *Induction of mitosis*

The results described give renewed confirmation to the view that the moulting hormone does not itself induce mitosis. It induces 'activation', and mitosis among the

activated cells is then determined by their own habits as defined by the pattern of differentiation in the body. The nuclei in the epidermis have a certain standard density per unit area. When they are too sparse, as in the insect stretched by feeding, mitosis occurs and continues until the normal density is restored. When nuclei are close together, as in the unfed insect, activation may go on to moulting and cuticle formation without any mitosis at all.

It has long been noted that the haemocytes will continue to multiply in the non-moulting insect. It was interesting to find during the present experiments that 'activation' in the fat-body cells is likewise independent of the moulting hormone. Fat-body cells, exposed to nutrients alone, become activated, and if their nuclei are widely separated mitosis takes place and continues until the normal nuclear density is restored.

(iii) *Action of the juvenile hormone*

The juvenile hormone and the moulting hormone are not antagonistic in their action as they are sometimes reputed to be; they do two quite different things. The moulting hormone induces the activation of the epidermal cells that is necessary for growth. The nature of that growth is determined by the epidermal cells themselves.

The genes in the epidermal cells are subject to a pattern of determination which defines the form of the body. The nature of this pattern is unknown; but in the larval stages of a hemimetabolous insect such as *Rhodnius* the epidermal cells carry a dual pattern: a visible larval pattern and a latent, invisible, adult pattern (Wigglesworth, 1940). The juvenile hormone merely ensures (perhaps by some action at the level of the genes (Wigglesworth, 1954), perhaps indirectly by some action upon the cytoplasm (Wigglesworth, 1953)), that the larval pattern is maintained among the activated epidermal cells.

In the experiments described in this paper, this action of the juvenile hormone is illustrated only in its simplest form; that is, in the greater density of nuclei in the abdominal epidermis of the larval stages, and the consequent effects of the juvenile hormone in favouring cell multiplication—an effect which is the direct opposite of the more familiar effect of the juvenile hormone elsewhere in the body in suppressing cell multiplication and so preventing the outgrowth of adult wings and genitalia.

SUMMARY

1. The effects of prolonged fasting on the epidermal cells, ventral abdominal muscles and fat body of *Rhodnius* 4th-stage larvae are described. Ribonucleic acid (RNA) almost disappears from nucleolus and cytoplasm, and the mitochondria are reduced in numbers. Dark osmiophil deposits, believed to be breakdown products of mitochondria, appear in the cytoplasm.

2. The effects of nutrition, distension, and moulting hormone were studied on these three tissues after starvation.

3. Nutrition alone has little effect on the epidermal cells and ventral muscles; but nutrition plus moulting hormone at once restores their capacity for protein synthesis and growth, as shown by the enlargement of the nucleoli, the appearance of RNA in the cytoplasm, and a great increase in the mitochondria. This general effect is termed 'activation'; it leads on to cuticle formation and moulting.

4. In the cells of the fat body, 'activation' with similar changes in nucleolus, RNA content and mitochondria, together with storage of neutral fat and glycogen, are brought about by nutrition alone, in the absence of moulting hormone.

5. Mitosis is not a direct effect of the moulting hormone; it is evoked by the mutual separation of activated cells. Since (in the absence of injury) moulting hormone is needed for activation of the nuclei in epidermal cells and ventral muscles, mitosis in these cells can occur only after exposure to the hormone. But since the nuclei of the fat body are activated by nutrition alone, they will undergo mitosis in the absence of moulting—provided the nuclei are widely separated from one another.

6. The nature of the action of moulting hormone and juvenile hormone in the epidermal cells is discussed.

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