

MITOSIS AND THE CELL CYCLE IN THE METAMORPHIC MOULT OF THE MILK- WEED BUG, *ONCOPELTUS FASCIATUS*. A RADIOAUTOGRAPHIC STUDY

P. A. LAWRENCE*

Department of Biology, University of Virginia, Charlottesville, Virginia,
and Developmental Biology Center, Western Reserve University,
Cleveland, Ohio, U.S.A.

SUMMARY

A simple method of whole-mount radioautography is devised to investigate aspects of the cell cycle during metamorphosis in the epidermis of the milkweed bug, *Oncopeltus*. Tritiated thymidine is used to indicate DNA synthesis. As the label only lasts in the insect for about 2 h, a wave of labelled cells passes through the different phases of the cell cycle. The *S* period is found to overlap with an exceptionally long prophase and there is thus no G_2 period. The length of prophase (408 ± 10 min) is estimated from a plot of the fraction of labelled prophases against time after injection of label. By an equivalent method the length of the *S* period is found to be 289 ± 12 min. No labelled cells divide again until about 24 h after the previous mitosis, when some cells embark on a second mitosis. The minimum interphase (G_1) period is approximately 16 h. In the area studied, the cell number more than doubles during the proliferative mitoses; and it is thus possible, but not certain, that every cell divides at least once.

Fifth-stage larvae injected during the differentiative divisions (which are involved in the development of dense hairs) show that each of the three kinds of differentiative divisions has its own peculiar timing. The timing of the very first division, that of the epidermal cell which will become the hair mother cell, suggests that the cell is already different from its progenitors prior to prophase.

INTRODUCTION

Wigglesworth's studies (1933, 1937, 1940, 1963) on the epidermis of *Rhodnius* have painted a detailed picture of the normal course of mitotic events during the larval and metamorphic moults. Wigglesworth (1964) has also shown that epidermal mitoses are in part a homeostatic response to cell separation. Where cells are dense, as in an unfed *Rhodnius* caused to moult by parabiosis, many epidermal cells survive from one larval instar to the next, and appear to undergo metamorphosis without cell division (Wigglesworth, 1942).

The likelihood of observing any mitoses depends on the length of the mitotic process itself, and with the exception of the grasshopper neuroblast (Carlson & Hollaender, 1948) and the *Drosophila* embryo (Huettnner, 1933; Rabinowitz, 1941) little is known of this or other details of the somatic cell cycle in any insect. Some information on the cell cycle in moulting 5th-stage larvae of the Lygaeid bug, *Oncopeltus*, is presented in

* Present address: Department of Genetics, Milton Road, Cambridge.

this paper. Observations have been made on the time course of the differentiative divisions concerned in the development of hairs (compare Lawrence, 1966).

MATERIAL AND METHODS

A stock culture of *Oncopeltus fasciatus* Dall was maintained in a dark incubator at 29.5 ± 0.5 °C. At this temperature the 5th-larval stage lasts for approximately 150 h.

Whole-mount radioautography

Fifth-stage larvae (wt 50–70 mg) were injected through the metathoracic legs with $1 \mu\text{c}$ (6.05 c/mm , Schwarz Bioresarch) of tritiated thymidine in $1 \mu\text{l}$ of distilled water. The injection was performed at room temperature, and the insects were then returned to the incubator at 29.5 °C to be kept for fixation at various times. This treatment had no detectable effects on control insects, which developed and reproduced normally. No attempt was made to control for possible circadian rhythms in cell cycles, but consistent results were obtained with material fixed at very different times of the day.

The abdominal integument was separated from viscera and fat body, and the basement membrane removed. The integument was then fixed for 1 h in Carnoy's fluid, hydrated and after warming in water, was dipped once into Kodak NTB 3 emulsion. The integument was then placed on a metal mesh for desiccation and exposure for 2 weeks. Finally, the preparation was developed and fixed, stained in Hansen's trioxyaematein for 1 min, dehydrated and mounted. Because of local separation between emulsion and epidermis, labelling was not always present over all the integument; however, large areas of consistently labelled nuclei were found (Fig. 7). The labelling was usually light enough to permit detailed examination of the nucleus under the exposed emulsion and background was typically very low (about 3 grains per nucleus). Sometimes in areas of thick emulsion, the background was high, but no areas were used for counts where this could give rise to any doubts as to the status of nuclei. Labelling was found only over nuclei, or when over metaphase or telophase figures, only over chromosomes (Fig. 8).

The recognition of prophase and telophase is necessarily somewhat subjective. Nuclei were scored as being in prophase when the chromosomes were visible as fine threads, and the nuclei appeared granular in optical section. Often the chromatin was shrunken like a ball of wool and was surrounded by a clear halo (*p*, Fig. 9). In fact, 90% of prophases were in this condition, and only the remaining 10% could be classed as late prophases; that is the chromosomes were relatively short and fat and visible as individual organelles (*p*, Fig. 10A).

During the moult the cell number is increased by a wave of proliferative divisions, which begin at about 40 h after the previous ecdysis, peak at 50 h when the mitotic index may reach 20%, and continue until 90 h. These divisions were studied in insects injected at about 50 h after ecdysis. The differentiative divisions, which are found from 70 to 95 h, were studied in insects injected at about 80 h. These peak times were chosen to ensure, as far as possible, even distribution of cells throughout the various phases of the cell cycle.

Measurement of prophase and S by proportion

The estimation of prophase and period of DNA synthesis (*S* period) from fractions of labelled cells measured at different times after injection of label is possible because, in *Oncopeltus*, these periods coincide for part of their duration. Two assumptions were made: (i) that the length of prophase is constant in the animals tested; and (ii) that the rate of cells entering prophase and the *S* period is constant for *each* of the insects over the duration of the labelling time.

As long as label is available, let *p* be the proportion of prophase cells that are labelled, *A* be the length of prophase (a constant), *S_p* be the length of time that prophase overlaps with the *S* period (a constant), and *t* be the time between injection of label and fixation. Then *p* is given by

$$p = \frac{S_p + t}{A}, \quad \left. \begin{array}{l} \text{as long as } t \leq A - S_p; \text{ and } p = 1, \text{ when } t \geq A - S_p. \end{array} \right\} \text{(see Fig. 1)} \quad (1)$$

Thus when *p* is plotted against *t* the graph will be a straight line with the intercept at *t* = 0 of *S_p/A*, and slope of 1/*A*. *S_p/A* and 1/*A* can be estimated from the linear regression of *p* on *t* and thus *S_p* and *A* can be calculated.

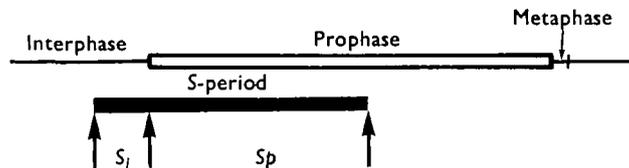


Fig. 1. The different periods in mitosis.

Similarly, when the label runs out and unlabelled cells begin to enter prophase, at time *t*₁, *p* is given by

$$p = \frac{A - (t - t_1)}{A} \quad \text{as long as } t > t_1 \geq A - S_p. \quad (2)$$

Hence from equations (1) and (2), when the label is no longer available, and unlabelled prophase cells are now appearing once again, the negative slope, 1/*A*, is the same as the positive slope when the label is available.

The mean time, *t*₁, at which unlabelled cells begin to enter prophase, is given by the intersection of equations (1) and (2).

As the *S* period also overlaps with interphase a comparable method can be used to estimate the period of overlap and hence the length of the *S* period itself.

If *q* is the true proportion of labelled cells that are in interphase and *S_i* is the length of time that interphase overlaps with the DNA synthetic or *S* period (a constant), as *S* = *S_p* + *S_i*, *q* is given by

$$q = \frac{S_i}{S + t} = \frac{S_i}{S_i + S_p + t} \quad \text{(see Fig. 3)}$$

therefore

$$\frac{1}{q} = 1 + \frac{(S_p + t)}{S_i}. \quad (3)$$

Unfortunately, when readings of the proportion of labelled interphases were made it was found impossible to classify some cells with certainty. A method was therefore devised by Dr John Stewart of the Department of Genetics, Cambridge, to make the best estimates from this data. Suppose that the labelled cells are divided into three groups: let q_s equal the proportion of labelled cells certainly in interphase; let q_u equal the proportion of cells for which classification is uncertain; then $1 - (q_s + q_u)$ is the proportion of cells seen to be in prophase.

In this case $1/q$ will lie between $1/q_s$ and $1/(q_s + q_u)$.

Suppose that

$$\frac{1}{q} = r, \quad \frac{1}{q_s} = r_s \quad \text{and} \quad \frac{1}{q_s + q_u} = r_u.$$

We may then suppose that $r = r_s + u(r_u - r_s)$, where u is a constant between 0 and 1, and is related to the proportion of unclassifiable cells which are in fact interphase. Hence from equation (3):

$$\begin{aligned} 1/q &= r = r_s + u(r_u - r_s) \\ &= 1 + (S_p + t) \times 1/S_i. \end{aligned}$$

Therefore

$$\frac{r_s - 1}{S_p + t} = \frac{1}{S_i} + \frac{u(r_s - r_u)}{S_p + t}; \quad (4)$$

therefore

$$\frac{r_s - 1}{S_p + t} \text{ plotted against } \frac{r_s - r_u}{S_p + t}$$

will be a straight line with intercept $1/S_i$ and slope of u . From the regression equations $1/S_i$ and u can be estimated. The value of S_p is calculated from equations (1) and (2) and substituted in (4).

Cell counts

A tergal area was chosen for this quantitative study (the central region of the third abdominal tergite) because, not only does it show no special growth associated with metamorphosis, but also the adult has very few hairs in this region. The average cell density was measured in individuals fixed before (0 h) and after (100 h) cell division, by counting cells in fields selected at random within the area. To correct for cuticular expansion, which occurs throughout the growth period, the third tergites of 20 5th-stage larvae were drawn with the camera lucida before and after proliferative cell divisions. The average increase in this area was found to be $54 \pm 2\%$.

Confidence limits

When confidence limits are given they represent the standard error of the mean.

RESULTS

Relative increase in cell number of tergites

The cell density was found to increase from $10800 \pm 65/\text{mm}^2$ to $15200 \pm 100/\text{mm}^2$, which, when corrected for cuticular expansion of this area ($54 \pm 2\%$) amounted to a relative increase of cell number from 1 to 2.17. As the only dying cells seen in insects

fixed throughout the mitotic period were invariably associated with differentiating bristles or hairs this ratio can be used to estimate the number of mitoses. Each tergal cell therefore undergoes an average of about one division during the metamorphic moult. It is convenient that in *Oncopeltus*, unlike *Rhodnius* (Wigglesworth, 1942), most cell death occurs close to, or after, the adult ecdysis.

Mitotic index

A quantitative survey of the mitotic index in samples fixed at different ages was made (compare Guillaume, 1961). This study gave no information about the real variation of the mitotic index during development of an individual, because of the large and unknown variance in the developmental states of the insects used. This problem can only be overcome in relatively synchronous cultures of known variance in developmental stage.

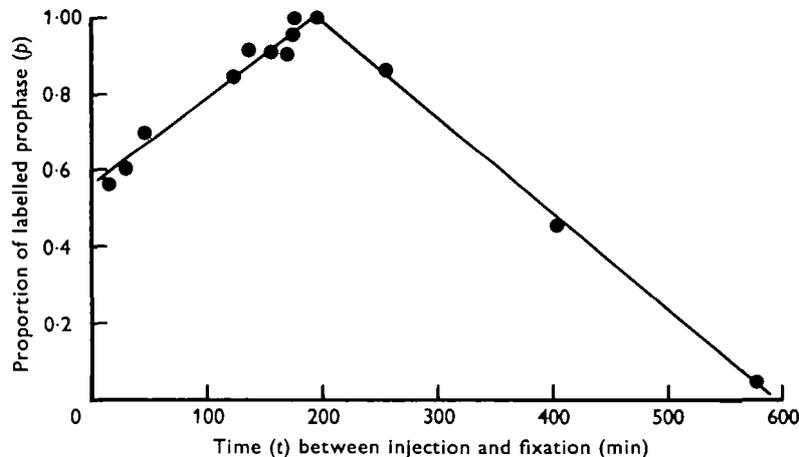


Fig. 2. The proportion of labelled prophases from all prophases against time after injection. The slopes for the rising and falling parts of the graph were calculated from the regression equations (the two points of complete prophase labelling were used in both calculations). Each point was derived from counts made on two or three insects.

The length of prophase

Insects fixed at various times between 15 and 180 min after injection of thymidine had label only over interphase and prophase nuclei. Figure 2 plots the proportion of all prophase which are labelled against time after injection of labelled thymidine. The very high proportion of labelled prophase cells after only 15 min, and the slow rise of the graph show that a considerable fraction of prophases are incorporating tritiated thymidine. The *S* period thus overlaps into prophase. If prophase is of one length in all the animals tested and if there are no sudden fluctuations in the rate of entry of cells into prophase in each of the experimental insects, then the proportion of labelled prophases should rise linearly. The points do fall on a good straight line.

By 180 min all the prophases are labelled in some groups. The proportion of labelled prophases begins to fall once again at a linear rate: the tritiated thymidine

is now no longer effectively available and unlabelled cells are now entering prophase as labelled cells leave it. The slopes of the ascending and descending portions of the graph should be the same within experimental error, and indeed by calculation were found to be 0.234 ± 0.017 and 0.247 ± 0.007 respectively. These two slopes were therefore pooled and the result (0.245 ± 0.006) used to calculate the length of prophase, which is equal to the mean time taken for the proportion of labelled prophase cells to rise from 0 to 1, and was found to be 408 ± 10 min (see Methods).

Figure 2 can also be used to calculate the proportion of labelled prophases at zero time (55%) which is equal to the proportion of prophase which is in the S period. S_p is thus 226 ± 6 min.

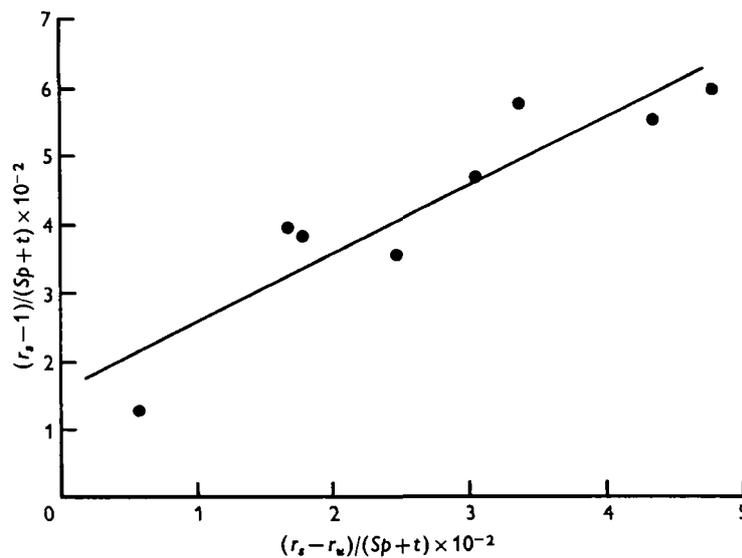


Fig. 3. Graph which allows the calculation of the constants S_i (period of DNA synthesis in interphase) and u (proportion of cells of doubtful classification which were, in fact, interphase). For derivation of this straight-line equation and meaning of symbols see Methods. Each point was derived from counts made on two or three insects.

As the G_2 in mammalian cells can be defined as the period intervening between the end of DNA synthesis and the beginning of cytologically recognizable prophase (compare Howard & Pelc, 1953) there is clearly no corresponding interval in these *Oncopeltus* cells.

The length of the S period

Similar measurements of proportion allow for an estimate of the length of S , as fortunately a small proportion of interphase cells also incorporate label. The certain distinction of interphase labelled cells (partly because of the overlying grains) was somewhat more difficult and cells were separated into three classes; labelled prophases, labelled interphases and labelled cells of uncertain stage. Using Stewart's formula (see Methods) it was possible to plot these figures in such a way as to allow for this uncertain class (Fig. 3) and make the best estimate of S from this data and from Fig. 2.

From Fig. 3, the intercept ($1/S_i$) was found to be $1.58 \pm 0.24 \cdot S_i$, the period of overlap of DNA synthesis into interphase, was therefore equal to 63 ± 10 min. DNA synthesis extends into prophase for the period S_p (226 ± 6 min). Adding S_i and S_p and pooling the errors, the S period emerges as being 289 ± 12 min long.

The slope (u) was calculated to be 1.00 ± 0.03 . There is thus internal evidence that all cells for which classification was difficult were in fact interphases. Rejection of this class of cells would therefore have given a serious underestimate of the true proportion of labelled interphases.

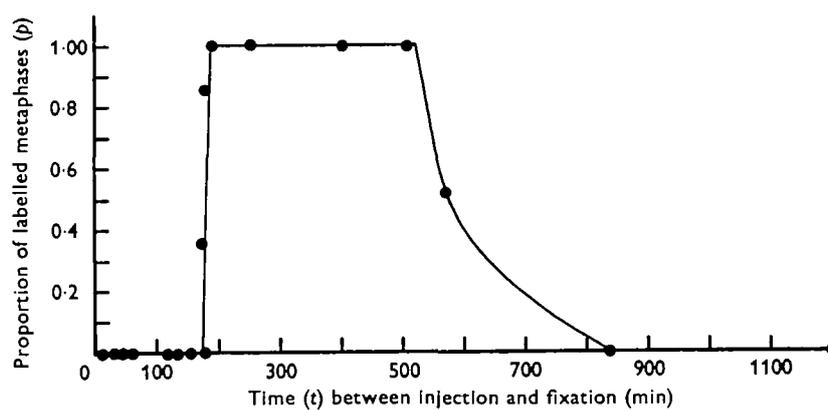


Fig. 4. Proportion of labelled metaphases against time after injection of label. The time between the 0.50 rising and falling sections of the graph is approximately equal to the length of the S period plus availability time of the thymidine. Each point was derived from counts made on two or three insects.

The figure of 289 min for the S period gains support from the proportion of labelled metaphases (Fig. 4). By 180 min after injection approximately 50% of metaphases are labelled. No metaphases are found labelled until 180 min, when the rise is extremely rapid. After about 540 min the proportion of metaphases begins to decline again, and reaches zero. The period intervening between the 50% point on the rising part of the curve and the 50% point on the falling part is equal to the length of S plus the availability time for thymidine (determined below as approximately 130 min). From Fig. 4 it is clear that this period is about 390 min which allows an estimate for S of about 260 min, which is quite close to the estimate by proportion.

Persistence of label in the insect

The time, t_i (see Methods) when unlabelled cells begin to enter prophase, was estimated from Fig. 2 as being 190 min. These unlabelled cells have already passed through the period of S in interphase (S_i) which is equal to 63 min. The label must have been available for approximately 130 min. During this time the thymidine is broken down or excreted by the insect. Coinciding with these proliferative divisions the insect is excreting urine at a high rate (Lawrence, 1965).

Metaphase, anaphase and telophase

The labelling of these short stages is not consistent. It is common to see labelled anaphases or sometimes even early telophases adjacent to unlabelled metaphases in preparations fixed at 180 min (compare Fig. 7). Clearly, the time taken for individual cells to pass through prophase varies. This inconsistency prohibits determination of the lengths of these short phases directly from the radioautographs. However, the mean length of prophase has been quite accurately determined as 408 ± 10 min, and this figure can be used to calculate the lengths of the other mitotic phases with some precision. From a count of randomly selected areas of control animals of 2500 mitotic cells 88.7% were in prophase, 2.9% were in metaphase, 2.3% in anaphase and 6.1% in telophase. Assuming random distribution of cells throughout the mitotic cycle, which is almost certainly achieved when such a large number is used, the lengths of metaphase, anaphase and telophase are 13, 11 and 28 min respectively. Mitosis thus takes an average of 460 min.

The length of interphase

In insects fixed between 10 and 20 h after injection all labelled cells are found to be in interphase pairs (Fig. 9), even though the proportion of dividing cells may reach 15%. Clearly the population of cells dividing at this time is distinct from the population which was in mitosis at the time of injection. The minimum time which may intervene between the end of *S* and the beginning of the next round of mitosis, is 20 h. The G_1 period is thus at least 15 h (Fig. 5). The G_1 period, although typical, is not immutable for, in a burnt area of an insect fixed 20 h after injection, where active wound-healing was present, labelled mitoses were seen.

Insects fixed at $22\frac{3}{4}$ h were different, and showed a large number of labelled prophases; no metaphase, anaphase or telophase stages were labelled. Sometimes both of a pair of labelled sister cells were in prophase, but more often one was in prophase and the other in interphase. These second divisions were particularly common in the intersegmental membranes; over the general surface of the segment most of the labelled cells remained in interphase.

The two groups of results restrict the normal minimum G_1 to between 15 h and $17\frac{3}{4}$ h, a conclusion which is confirmed by insects fixed 26 h after injection which showed all stages of mitosis labelled. As the *S* period overlaps into prophase for about $3\frac{3}{4}$ h the total cell cycle time estimated from these experiments must lie between $23\frac{3}{4}$ and $26\frac{1}{2}$ h. Information on the cell cycle is summarized in Fig. 5.

The presence of undivided pairs of cells in insects fixed at $22\frac{3}{4}$ h suggested that many cells do not pass through another cell cycle. This impression was substantiated by a group of insects injected at 41 h after moulting and fixed 44 h later. In this group, although some labelled cells were dividing, most labelled cells were in interphase, and many were still in isolated pairs. These pairs were the daughter cells of a single division, and as this experiment (injected 41 h, fixed 85 h after the moult) spans most of the time available for proliferative divisions, it suggests that many cells divide but once during the moult. This conclusion is consistent with the small total increase in

cell number during the proliferative cell divisions, and the lack of dying cells at this time. Clearly, other regions which exhibit considerable growth during the metamorphic moult, for instance the posterior margin of the third sternite of females, show a large multiplication of cell number and here consecutive cell divisions must occur. In slides prepared from animals 44 h after injection isolated pairs of labelled cells were not found in this area, and cell division persists here when it has ceased elsewhere in the abdomen.

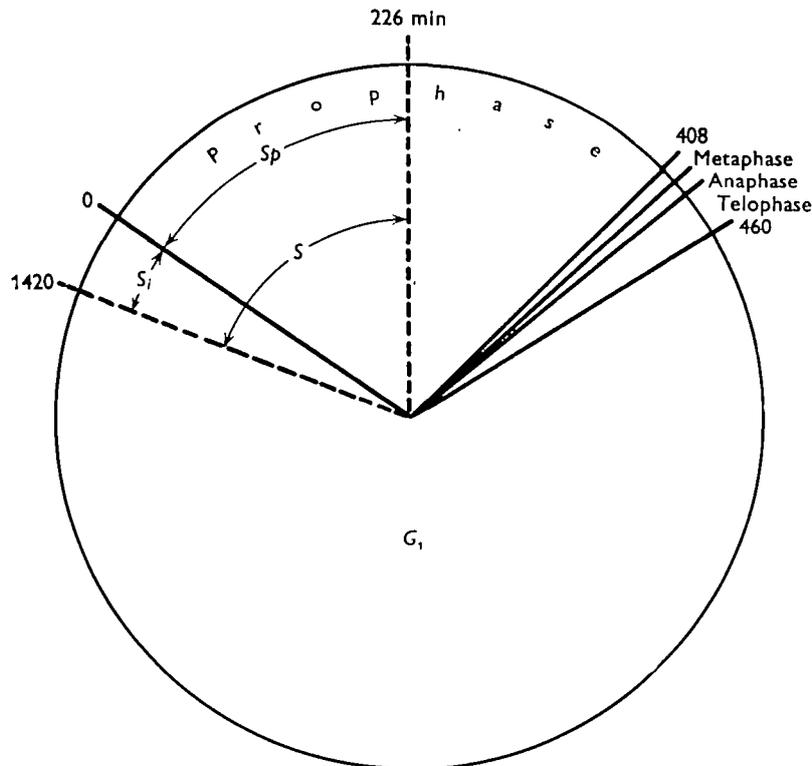


Fig. 5. Summary of cell cycle of the proliferative divisions.

Differentiative divisions

As the proliferative divisions wane, new types of cell divisions are found in the epidermis. These are the differentiative cell divisions involved in hair formation (Lawrence, 1966). Radioautographs prepared from insects labelled at about 70 h after the ecdysis, have revealed differences in the timing of these mitoses. Figure 6 summarizes the cell lineage of a hair.

In insects fixed at 120 min after injection, the epidermal cells and the hair mother cells I and II are labelled only in prophase, and interphase. However, the divisions of the accessory mother cell include labelled metaphases, anaphases and telophases. Certainly, the time intervening between *S* and telophase is much shorter for the vertical divisions of this cell.

Insects fixed at 135 min amplify the above conclusions; for some labelled accessory mother cells have now reached interphase, and, moreover, whereas the epidermal

cells are still labelled only in prophase and interphase, the hair mother cells II are labelled in metaphase. It appears therefore that the hair mother cells II divide somewhat more rapidly than either the epidermal cells or the hair mother cells I.

Insects fixed at 165 min (Fig. 10) have some of the hair mother cells I divisions labelled at metaphase and anaphase. In contrast, no metaphases of the proliferative epidermal cells are yet labelled. The hair mother cell I mitosis is therefore somewhat more rapid than the proliferative divisions.

Each of the differentiative divisions thus has a prophase of different and characteristic length.

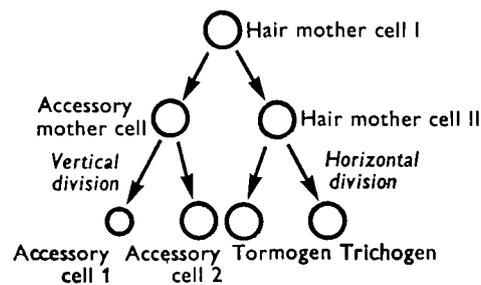


Fig. 6. Cell lineage of a hair.

Taken together these pieces of information suggest that the period intervening between the end of *S* and the termination of the mitosis of the hair mother cell I is about 3 h, then there is a period of DNA synthesis, which begins earlier in the accessory mother cell than in hair mother cell II and ends only 2 h 15 min before termination of the mitosis of the accessory mother cell and somewhat less than 3 h prior to the end of cell division of the hair mother cell II. Accurate estimation of the total length of this differentiative process is impossible from my data because of complete asynchrony of the cell population. However, the interphases between mitoses of these differentiating cells must be very short, of the order of 3 h, because very few groups containing even one interphase are found.

DISCUSSION

The full degree of variation of cell cycles is now being appreciated (Cleaver, 1967); even in the same organism at different stages of its life-history all phases of the cell cycle can alter in length (Graham & Morgan, 1966). Care must therefore be taken in arguing from one organism to another. Until more work is done on cell cycles in insects it will not be possible to decide whether the time course of cell division in *Oncopeltus* shares features with other insects.

The most striking characteristic of the cell cycle of *Oncopeltus* is the overlap of *S* into a very long prophase. DNA synthesis in prophase has been described in other organisms, for instance the embryonic neuroblasts of the grasshopper *Chortophaga* (Gaulden, 1956) and the microsporocytes of *Tradescantia* (Moses & Taylor, 1955). In the development of *Schistocerca*, although the neuroblasts show the overlap between

prophase and *S*, the other embryonic nuclei incorporate label only in interphase (S. A. Henderson, personal communication). In *Oncopeltus* the normal events of prophase occur after *S* is completed and continue at a rapid rate. Active contraction of chromosomes seems to be confined to the very beginning of prophase, thus rendering the chromosomes visible as fine threads, and to the last 10% (about 40 min) when the chromosomes become discernable as individual organelles.

Cell separation is an important trigger for mitosis in the epidermis of *Rhodnius* (Wigglesworth, 1964). One of its first effects would now seem to be DNA synthesis; because in *Oncopeltus*, at least, where there is no G_2 period, DNA synthesis is intimately linked with mitosis. Krishnakumaran, Oberlander & Schneiderman (1965) have suggested that DNA synthesis might be a direct response to ecdysone. But mitosis has been shown to be independent of ecdysone in the fat body of *Rhodnius* (Wigglesworth, 1963). Moreover, activation of the cells is the first observable effect of ecdysone (Wigglesworth, 1933) and occurs synchronously in all the epidermal cells. Divisions are asynchronous and seem unlikely to be a direct response to a circulating hormone which should affect all the cells evenly. Moreover Krishnakumaran, Berry, Oberlander & Schneiderman (1967) themselves have now found evidence for the independence of DNA synthesis from ecdysone.

The general course of mitotic events in *Oncopeltus* is similar to that in other insects. Some days after the initiation of the metamorphic moult, mitoses begin and persist for several days (Wigglesworth, 1940, 1948; Guillaume, 1961). They follow a pattern as certain areas, in particular the intersegmental membranes, begin sooner and often continue longer than other areas. Because of the large variation in growth of different parts of the insect the cell cycle would be expected to vary considerably. In *Oncopeltus* only the abdominal sternites and tergites have been studied, but within these regions various degrees of growth occur; for example in the posterior margin of the third sternite of females, which, owing to excessive cellular multiplication, expands over the anterior part of the fourth sternite. Here there is evidence that the essential features of the cell cycle remain the same as elsewhere on the sternites but that many more, if not all, the daughter cells proceed to at least a second division.

In this study it was hoped to determine whether all cells in the epidermis of *Oncopeltus* divide at the metamorphic moult; but unfortunately the tritiated thymidine does not remain long enough in the insect for this to be discovered. However, on the tergites where the cells increase more than 2 times, the same cells do not divide a second time for 24 h, and cell division only lasts for about 40 h, it is quite possible that every cell divides at least once—but this remains uncertain. Wigglesworth's experiments with moulting unfed 5th-stage larvae of *Rhodnius* suggest that cell division is not a prerequisite for metamorphosis. Here metamorphosis was accomplished with almost no change in cell number, and very few divisions were seen (Wigglesworth, 1942). It is worth reiterating, moreover, that in both *Rhodnius* and *Oncopeltus* the same bristle cells metamorphose to produce a different type of bristle without DNA synthesis or divisions (Wigglesworth, 1933; Lawrence, 1966).

It is not surprising that the three types of differentiative divisions have their own characteristic time courses. This seems to echo observations of Stebbins (1965), who

studied similar systems in plants, that the phases of the cell cycle differ in each type of division. In the case of hair mother cell I, the unique length of the prophase period implies that these cells are different prior to the mitosis itself—a property which is also apparent cytologically (Lawrence, 1966). The essential determinative process presumably occurs during the interphase (G_1) of the epidermal cell.

I am very grateful to Dr J. G. Kunkel and Dr J. Stewart for help with data analysis. Dr Stewart is responsible for the derivation of the equations in the methods section. I thank Professors D. Bodenstern, M. Locke and H. A. Schneiderman for the hospitality received in their laboratories, and acknowledge financial support from the Commonwealth Fund and grant G.M. 09960 of the U.S. Public Health, awarded to Dr Locke. Dr J. E. Cleaver and Dr J. G. M. Shire have read the manuscript and suggested improvements.

REFERENCES

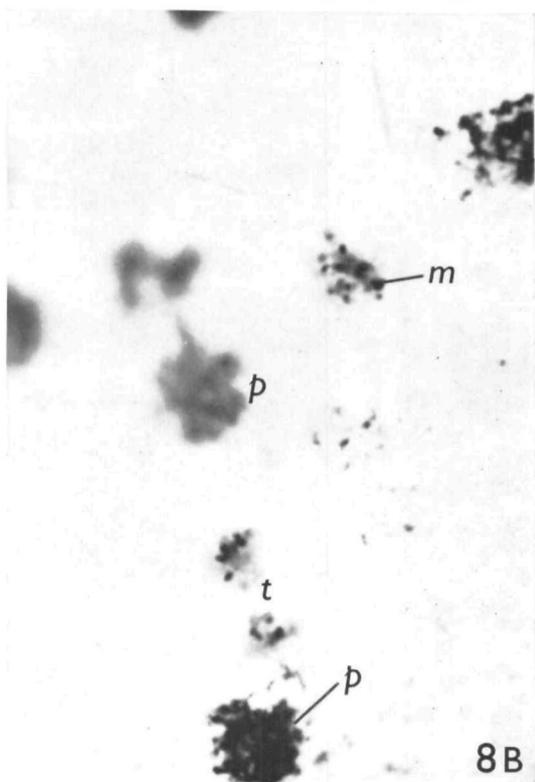
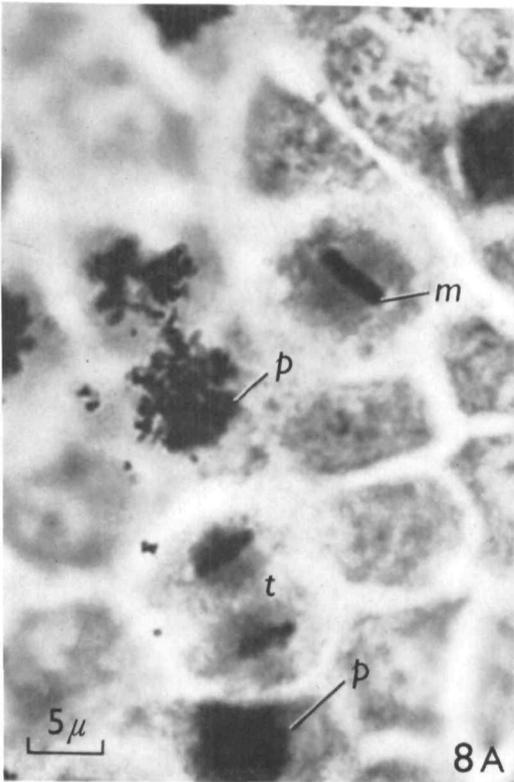
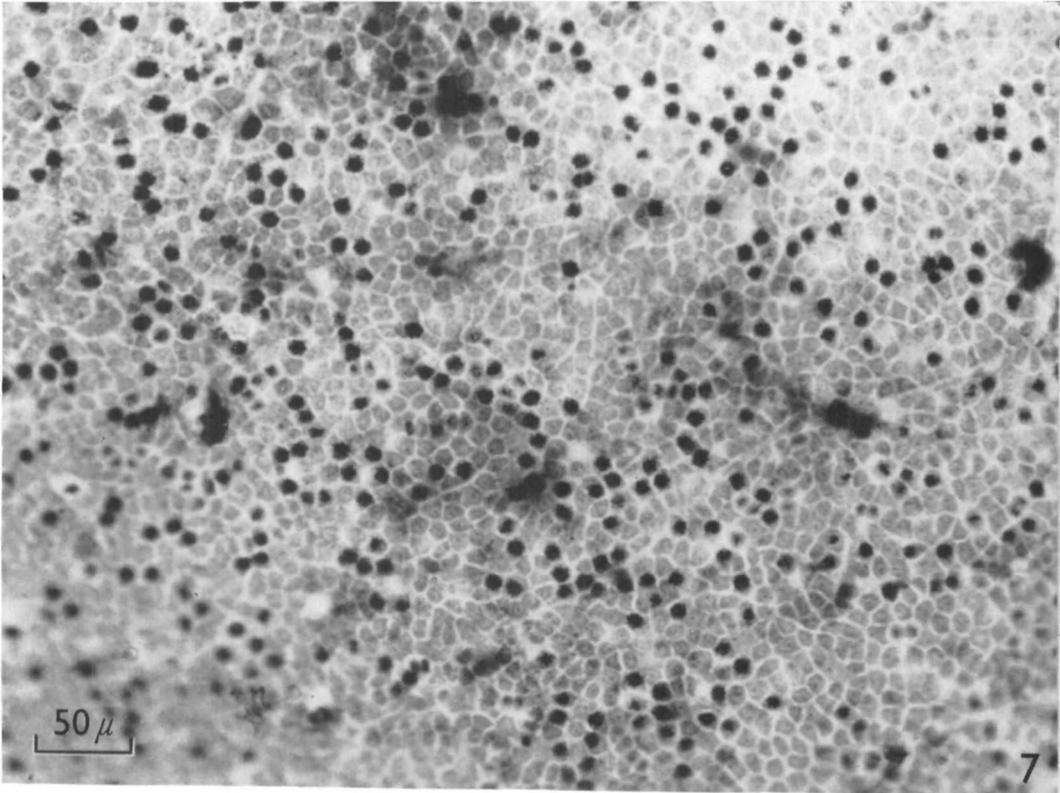
- CARLSON, J. G. & HOLLAENDER, A. (1948). Mitotic effects of ultraviolet radiation of the 2250 Å region, with special reference to the spindle and cleavage. *J. cell. comp. Physiol.* **31**, 149–173.
- CLEAVER, J. E. (1967). *Thymidine Metabolism and Cell Kinetics*. Amsterdam: North Holland Publishing Company.
- GAULDEN, M. E. (1956). DNA synthesis and X-ray effects at different mitotic stages in grasshopper neuroblasts. *Genetics* **41**, 645.
- GRAHAM, C. F. & MORGAN, R. W. (1966). Changes in the cell cycle during early amphibian development. *Dev. Biol.* **14**, 439–460.
- GUILLAUME, M. (1961). La multiplication cellulaire au cours l'intermue dans divers tissus du phasme *Clitumnus extradentatus* Br. *Bull. Soc. zool. Fr.* **86**, 361–371.
- HOWARD, A. & PELC, S. R. (1953). Synthesis of desoxyribonucleic acid in normal and irradiated cells and its relation to chromosome breakage. *Heredity* **6** (suppl.), 261–273.
- HUETTNER, A. F. (1933). Continuity of the centrioles in *Drosophila melanogaster*. *Z. Zellforsch. mikrosk. Anat.* **19**, 119–134.
- KRISHNAKUMARAN, A., BERRY, S. J., OBERLANDER, H. & SCHNEIDERMAN, H. A. (1967). Nucleic acid synthesis during insect development. II. Control of DNA synthesis in the Cecropia silkworm and other Saturniid moths. *J. Insect Physiol.* **13**, 1–57.
- KRISHNAKUMARAN, A., OBERLANDER, H. & SCHNEIDERMAN, H. A. (1965). Rates of DNA and RNA synthesis in various tissues during a larval moult cycle of *Samia cynthia ricini* (Lepidoptera). *Nature, Lond.* **205**, 1131–1132.
- LAWRENCE, P. A. (1965). *The Determination and Development of Hairs and Bristles in the Milkweed Bug, Oncopeltus fasciatus* Dall. Thesis, University of Cambridge.
- LAWRENCE, P. A. (1966). Development and determination of hairs and bristles in the milkweed bug, *Oncopeltus fasciatus* (Lygaeidae, Hemiptera) *J. Cell Sci.* **1**, 475–498.
- MOSES, M. J. & TAYLOR, J. H. (1955). Desoxypentose nucleic acid synthesis during microsporogenesis in *Tradescantia*. *Expl Cell Res.* **9**, 474–488.
- RABINOWITZ, M. (1941). Studies on the cytology and early embryology of the egg of *Drosophila melanogaster*. *J. Morph.* **69**, 1–49.
- STEBBINS, G. L. (1965). Some relationships between the mitotic rhythm, nucleic acid synthesis and morphogenesis in higher plants. *Brookhaven Symp. Biol.* **18**, 204–221.
- WIGGLESWORTH, V. B. (1933). The physiology of the cuticle and of ecdysis in *Rhodnius prolixus* (Triatomidae, Hemiptera); with special reference to the function of the oenocytes and of the dermal glands. *Q. Jl microsc. Sci.* **76**, 269–318.
- WIGGLESWORTH, V. B. (1937). Wound healing in an insect (*Rhodnius prolixus* Hemiptera). *J. exp. Biol.* **14**, 364–381.
- WIGGLESWORTH, V. B. (1940). The determination of characters at metamorphosis in *Rhodnius prolixus* (Hemiptera). *J. exp. Biol.* **17**, 201–222.
- WIGGLESWORTH, V. B. (1942). The significance of 'chromatic droplets' in the growth of insects. *Q. Jl microsc. Sci.* **83**, 141–152.

- WIGGLESWORTH, V. B. (1948). The functions of the corpus allatum in *Rhodnius prolixus* (Hemiptera). *J. exp. Biol.* **25**, 1-14.
- WIGGLESWORTH, V. B. (1963). The action of moulting hormone and juvenile hormone at the cellular level in *Rhodnius prolixus*. *J. exp. Biol.* **40**, 231-245.
- WIGGLESWORTH, V. B. (1964). Homeostasis in insect growth. *Symp. Soc. exp. Biol.* **18**, 265-281.

(Received 22 December 1967)

Fig. 7. Whole mount radioautograph of 5th-stage larva injected during proliferative mitoses and fixed after 180 min. Some of the metaphases, anaphases and telophases are labelled after this incubation period. Most labelled cells are in prophase.

Figs. 8A, B. Whole mount radioautograph of 5th-stage larva made during proliferative cell divisions and fixed 180 min after injection. Metaphase (*m*) and telophase (*t*) are pictured in focal plane of chromosomes (A) and the focal plane of grains (B). These mitotic figures are lightly labelled in contrast to prophase (*p*) because they are in the vanguard of the population of labelled cells.



P. A. LAWRENCE

(Facing p. 404)

Fig. 9. Whole mount radioautograph of 5th-stage larva injected at 43 h after the ecdysis and fixed 20 h later. The labelled cells are in interphase pairs (e.g. 1, 2, 3). Dividing cells in prophase (*p*) and telophase (*t*) are unlabelled.

Figs. 10A, B. Whole mount radioautograph of 5th-stage larva injected during early differentiative divisions and fixed 165 min later. A, in focal plane of nuclei; B, in focal plane of grains. Accessory mother cell is in a late telophase (*at*) which is oriented vertically to the surface plane of the cuticle and is labelled, as is the prophase of the hair mother cell II (*hIIp*), and hair mother cell I (*hIp*). In contrast, a later prophase (*p*) and a telophase (*t*) of proliferative cell divisions are unlabelled.

