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Cell Cycles in the Male Accessory Glands of Mealworm Pupae

GEORGE M. HAPP, BONNIE J. MACLEOD, TERESA M. SZOPA,[1] CONNIE S. BRICKER, THOMAS C. LOWELL, JORDAN H. SANKEL, AND CHRISTINE YUNCKER

Department of Zoology, University of Vermont, Burlington, Vermont 05405

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During the pupal stage of *Tenebrio molitor*, the accessory reproductive glands of males grow by cell division. Within the secretory epithelium of the bean-shaped accessory glands (BAGs), cell numbers triple. In the tubular accessory glands (TAGs), the increase is 14-fold. There are two mitotic maxima in each gland. The first maximum occurs at 1-2 days while the second is at 4-5 days. The second maximum coincides with the major ecdysteroid peak described by Delbecque *et al.* [*Dev. Biol.* **64**, 11-30 (1978)]. Nuclei were isolated from TAGs during the pupal mitotic bouts and during mitotic inactivity in the adult. After Feulgen or propidium iodide staining, the DNA content of these nuclear populations was measured by absorption cytophotometry or by fluorescence flow cytometry, respectively. The proportion of cells in each phase of the cycle was calculated using an iterative model. After mitoses have ended in the late pupa, the cells were arrested in G_2. [^{3}H]Thymidine was injected into 1- and 4-day pupae to pulse-label cells of the TAGs. After allowing various periods from 4 to 60 hr for cells to progress through G_2 to reach mitosis, fractions of labelled mitoses were determined by autoradiography. From the combined cytometric and autoradiographic data, the duration of each phase of the cell cycle was calculated assuming the population was in exponential growth. Cell cycles in 4-day pupal TAGs take 48 hr. G_1, S, G_2, and, M lasted 13, 14, 17, and 4 hr, respectively.

INTRODUCTION

Mitotic activity and a shift in the pattern of gene expression often take place simultaneously during development. According to the "quantal mitosis" hypothesis, this commonly observed coincidence of the two phenomena reflects an underlying requirement for a turn of the cell cycle before a new phenotype can be expressed; "only quantal cell cycles are believed to lead to rearrangements in chromosomal structure required to reprogram daughter cells" (Holtzer *et al.*, 1972). This hypothesis has been supported by extensive studies of myogenesis, erythrogenesis, and chondrogenesis in mammalian and avian material (Holtzer *et al.*, 1972). Similar correlations between developmental reprogramming cell cycles were also suggested for the epidermis of insects (Kühn and Piepho, 1938; Schneiderman, 1972; Willis, 1974). But, subsequent studies of cells and tissues from mammalian (Nadal-Ginard, 1978; Levenson *et al.*, 1980) and insect sources (Selman and Kafatos, 1974; Kumaran, 1978; Dyer *et al.*, 1981; Hakim, 1982) have indicated that there are significant exceptions to Holtzer's intriguing hypothesis. We propose to evaluate the linkage between cell cycles and reprogramming in a new model—the accessory reproductive glands of a male beetle.

There are two pairs of accessory glands in male mealworm beetles (*Tenebrio molitor*). The products of both pairs make up the spermatophore, an elaborate, organized sac that packages sperm for transfer to the female. The larger bean-shaped accessory glands (BAGs) manufacture a semisolid secretory mass that is molded into the wall of the spermatophore (Frenk and Happ, 1976; Dailey *et al.*, 1980). The smaller tubular accessory glands (TAGs) secrete soluble proteins which mingle with the sperm (Black *et al.*, 1982; Black and Happ, 1984).

Primary organogenesis of the BAGs and the TAGs has been accomplished by the time of pupal ecdysis (Huet, 1966). Maturation of these glands takes place during the pupal stage and the first week of adult life, during which time the protein content of both organs increase 15-fold (Happ *et al.*, 1982; Happ and Happ, 1982). Mitotic figures are common during the first 7 days of the 9-day pupal stage (Grimes and Happ, 1980; Happ and Happ, 1982).

During the period when the accessory glands of *Tenebrio* are growing so rapidly, both radioimmunoassay and mass spectroscopy have shown that there are pronounced changes in the titers of ecdysteroids (Delbecque, 1975; Delbecque *et al.*, 1978; Delachambre *et al.*, 1980). Mitoses in the TAG and the BAG begin early in the pupal stage when ecdysterone levels are low, and cell division continues through the rising phase of the major ecdysteroid peak in the mid-pupa. As ecdy-

[1] Present address: Department of Biochemistry, The London Hospital Medical College, Turner Street, London E1 2 AD, England.

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steroid levels drop, mitotic activity wanes (Grimes and Happ, 1980, Happ and Happ, 1982). Shortly thereafter, the glands begin to produce their characteristic secretory products (Gadzama *et al.*, 1977; Happ *et al.*, 1977; Grimes and Happ, 1980; Happ and Happ, 1982; Black *et al.*, 1982; Happ *et al.*, 1982; Dailey and Happ, 1983).

In the present paper, we report changes in cell numbers and in mitotic frequencies during the maturation of the TAGs and the BAGs. Then we present data on the distribution of cells between G_1 and G_2 phases and we calculate the length of each phase of the cell cycle in the TAG.

MATERIALS AND METHODS

Animals. Mealworms (*T. molitor* L.) were obtained from commercial sources and also from laboratory colonies at the University of Vermont. Freshly eclosed pupae were sexed and allowed to develop at 26°C until used for experiments. Adults were similarly collected and maintained. Colonies were fed Purina Chick Labchow and potato or carrot.

Cell counts. Cell numbers in TAGs and BAGs were estimated by counting nuclei using a modification of the technique of Selman and Kafatos (1975). Glands were dissected in phosphate-buffered saline (PBS) (Dulbecco and Vogt, 1954) and transferred to 0.5 ml 20% sucrose in HEK-Mg buffer (0.01 *M* HEPES-KOH buffer, pH 7.4, 0.01 *M* KCl, 1 μM $MgSO_4$) in a Dounce tissue grinder and allowed to stand for 3 min. Buffer (5.75 ml HEK-Mg, without sucrose) was added and the glands were allowed to stand for 5 min. Then they were homogenized by seven strokes of the loose-fitting B pestle followed by two strokes of the tight-fitting A pestle. A 0.5-ml volume of 5.3% Tween 40 was added and the mixture stirred with two strokes of the A pestle. A 0.5-ml volume of 0.77% Azure blue B was added, and aliquots were counted in a hemocytometer.

Mitotic indices. Pupae were injected with 3 μl 1% colchicine in double-distilled water between the last two ventral sclerites, using a Hamilton syringe. After 2 hr at 25°C the gland complexes were dissected out and immediately fixed in ethanol:acetic acid (3:1) for 2 hr at room temperatures. Upon transfer to 45% lactic acid for 5–10 min, the BAGs and TAGs were dissected from each other. Each gland was placed on a microscope slide, excess lactic acid was blotted away, and the tissue was flooded with acetoorcein stain. A squash preparation was made by covering the tissue with a coverslip and applying moderate pressure for a few seconds. Excess stain was blotted away and the coverslips were sealed with molten petroleum jelly. They were allowed to stain overnight, and then counted.

Cytophotometry. Glands were dissected and nuclei isolated as described above for nuclear counts. After addition of Tween 40, the mixture was transferred to a Corex tube, an equal volume of sucrose–formalin (4% formaldehyde in PBS, 0.25 *M* sucrose) was added, and the nuclei were fixed overnight at room temperature. The fixed nuclei were pelleted (3000*g*, 12 min, 4°C), the supernatant was discarded, and 70% ethanol was added for 30 min. The ethanol was removed and 5 *N* HCl was added for 1 hr of hydrolysis at room temperature (22°C). The acid was removed, the pellet was rinsed in distilled water, and Schiff's reagent (Sigma 5-5113) was added for 30 min. The supernatant (Schiff's) was discarded, and the pellet was washed twice in 10% $K_2S_2O_5$ and then twice more with distilled water. The pellet was dehydrated through an ethanol series (30, 50, 70, and 100%), cleared for 5 min in xylene, and then stored in immersion oil.

Small portions of the pellet were placed on a slide and examined in immersion oil. To minimize light scattering during measurement of absorbance, we used immersion oil with NA = 1.550. The microscope was a Zeiss Ultraphot II, equipped with a MPMO1K photometer-photomultiplier and Zonax microcomputer.

Absorption was measured at two wavelengths (560 and either 510 or 500 nm) according to the general methods of Ornstein (1952) and Patau (1952). The shorter wavelength was selected to give a ratio of absorbance between longer and shorter wavelengths of between 1.8 and 2. The measuring diaphragm was 3.2 μm for pupal nuclei and 5 μm for adult nuclei.

The Zonax software records an average of 15 absorption readings at each wavelength. A short program in BASIC was written to allow computation of the DNA "machine units" from the two absorption means according to the formula presented in Rasch and Rasch (1970). For each experimental group at least 150 nuclei were measured. Each data set was normalized to a value of 4 machine units for 2C DNA and an overall distribution width of approximately 8 machine units.

Flow cytometry. The techniques for flow cytometry were patterned after those of Vindeløv *et al.* (1983), with increased concentrations of ribonuclease and the omission of trypsin. The citrate buffer contained 250 m*M* sucrose, 40 m*M* trisodium citrate, and 0.05% (v/v) dimethyl sulfoxide at pH 7.6. The stock solution contained 3.4 m*M* trisodium citrate and 0.1% (v/v) Nonidet P-40, at pH 7.6. Pancreatic ribonuclease (1 mg/ml, Sigma Chemical) was dissolved in stock solution. Propidium iodide (0.42 mg/ml, Sigma Chemical) was dissolved in stock solution and aliquots were frozen at −80°C until used for staining.

Glands were dissected as for cytophotometry, and the cells were dispersed in 200 μl citrate buffer by passing them several times through a 20- and then a

23-gauge needle. This cell suspension (either fresh or frozen) was mixed with 1.8 ml of stock solution for 10 min. A 1.5-ml volume of the ribonuclease solution was then added, and the two solutions were mixed for another 10 min. Finally, 1.5 ml of ice-cold propidium iodide in stock solution was added and, after mixing, the sample was filtered through a 20-μm nylon mesh (Tetko Corporation) and stored on ice until analysis, which took place 15 to 180 min after addition of stain. The flow cytometer was an Ortho Model 50H dual-laser cytoflorograph with a Model 2150 data handling system.

The proportion of cells in G_1 and $G_2 + M$ were determined by two computer programs supplied by Ortho. The simplest was the "Quickestimate." The Quickestimate algorithm assumes (a) that the G_1 and $G_2 + M$ peaks are symmetrical, and (b) that the region of the cell cycle histogram from the lower limit of analysis to the mean of the G_1 peak and the region from the mean of the G_2 peak to the upper limit of analysis contain no S-phase cells. For cells from 1- and 4-day pupal TAGs, the distribution of cells over the cell cycle was determined by an iterative method (Marquardt, 1963). G_1 and $G_2 + M$ are modeled by normal distributions and the S phase by a family of polynomial (quadratic) distribution (Gray and Dean, 1980). The computer program begins with an initial estimate of the fitting parameters (Dean, 1980), and then the parameters are adjusted simultaneously in an iterative fashion in order to produce a nonlinear least-squares fit and to minimize the residual sum squared error (Marquardt, 1963). For each analysis, the peak fluorescence areas (DNA content) of 10,000 cells were tallied.

Determination of fraction of labeled mitosis. The technique for fractions of labeled mitosis (FLM) was an adaptation of the approach of Quastler and Sherman (1959). [^{3}H]Thymidine (1 μCi; sp act, 90 Ci/mmole) was injected into the hemocoel of pupae of known ages. At various times after the [^{3}H]thymidine pulse, 1 μl 4% colchicine was similarly injected. Four hours later, the experiment was terminated. The glands were removed from the animals and fixed in ethanol:acetic acid (3:1) for 1 hr at room temperature. The tissues were transferred to 45% aqueous lactic acid for 5 min and then to acetoorcein (1 g orcein in 120 ml 9 *N* acetic acid), and were left overnight. Glands were squashed on a slide in a drop of stain. The slide was placed on a block of dry ice; its coverslip was pried off, and it was dried on a warming table. The dry slide was placed in Carnoy's for 10 min and hydrated. After air-drying, it was dipped in emulsion (5 ml Kodak NTB 2 emulsion, 5 ml 0.1% Tween 80), dried, and allowed to expose for 2–4 weeks.

After photographic development, at least 1000 nuclei of each slide were examined and scored as labeled or unlabeled and as mitotic or interphase. A minimum of six slides was scored for each experiment for these counts. The labeling index, mitotic index, and fraction of labeled mitoses were calculated.

RESULTS

Cell Numbers Increase in the TAG More Than in the BAG during the Pupal Stage

Cell numbers in BAGs and TAGs were estimated by dissecting each gland free of other organs, isolating the nuclei, and counting them in a hemocytometer. The results are shown in Fig. 1. At pupal ecdysis, each pair of BAGs contained 2.4×10^5 cells and the numbers increased to 7.5×10^5 cells 8 days later. The corresponding counts for the TAGs were 2×10^4 cells at ecdysis and 2.8×10^5 cells at the end of the pupal stage. On the assumption that cell growth is exponential, we can estimate the average doubling time from these data. According to the slope of the increase in cell numbers (Fig. 1), the doubling time for cells of the BAGs is 120.4 hr while that for the TAGs is 46.4 hr.

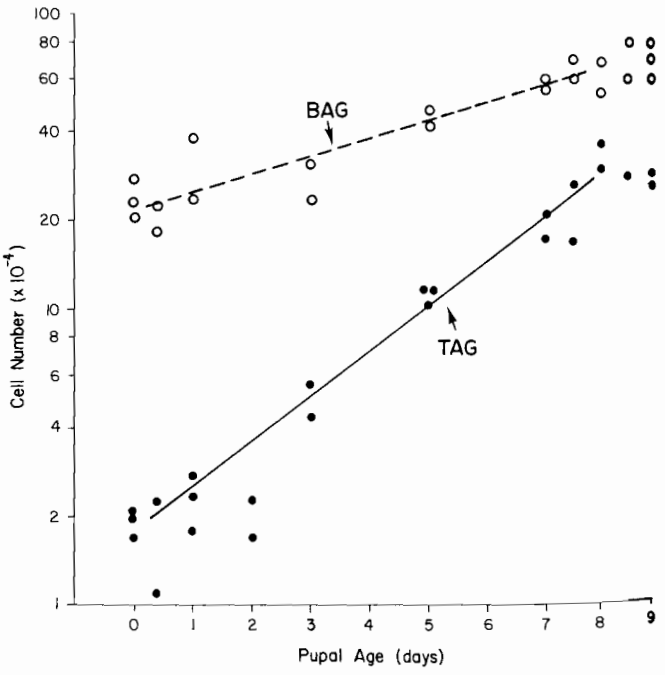

FIG. 1. The numbers of nuclei in each pair of the TAGs and the BAGs at various pupal ages. For the TAGs (solid line), the regression equation is $\log y = 0.155x + 4.23$, $r = 0.978$. For the BAGs (dashed line), the regression equation is $\log y = 0.06x + 5.34$, $r = 0.938$. Regression equations were calculated for the period of 0–8 pupal days; thereafter, cell number is constant.

Two Maxima in Mitotic Activity Take Place in the Pupal Stage

Mitotic indices were determined in TAGs and BAGs from pupae of various ages that had been injected with colchicine 2 hr before fixation (Fig. 2). There are two maxima in the mitotic indices. The first peak occurs on Days 1-2 and then cell division declines somewhat, only to rise again and to reach a second peak on pupal Days 4-5. By the eighth day, mitoses are very rare and we have never seen mitotic figures in postecdysial adults. This pattern agrees with earlier studies which used a 4-hr colchicine arrest (Grimes and Happ, 1980; Happ and Happ, 1982).

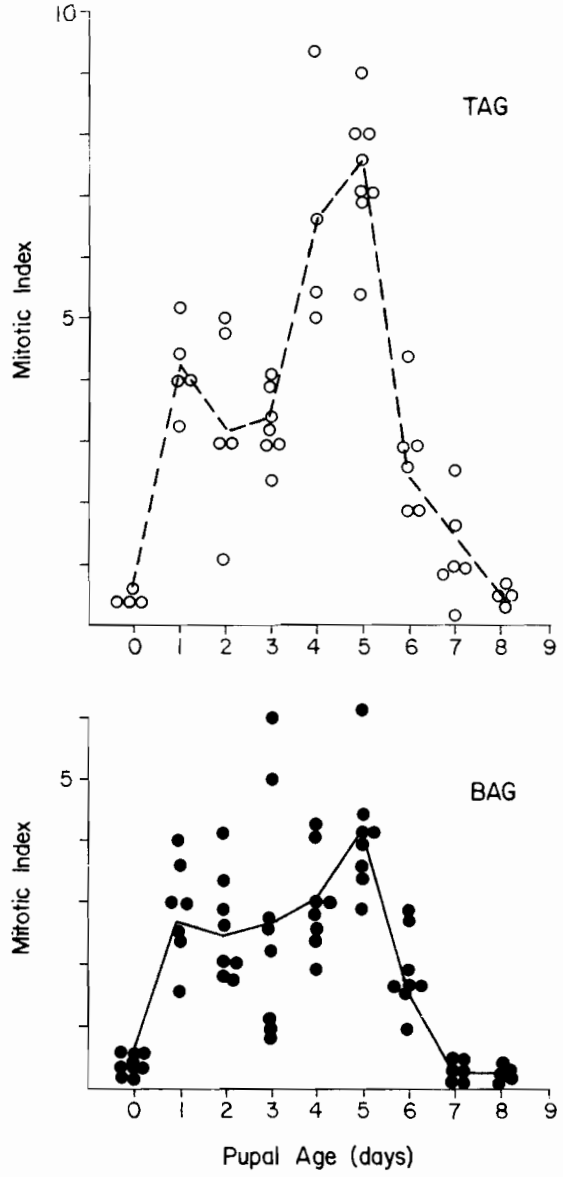

FIG. 2. Mitotic indices in the TAGs and the BAGs of pupae. Colchicine was injected into the hemocoel 2 hr before the glands were dissected, fixed, and squashed. The points represent counts of 500 cells. Lines connect the means of the counts for each age.

TABLE 1
MITOTIC INDICES OF TAGS

Ages at arrest[a]	Number	Mitotic index Mean %[b]	± SE
28-72 hr pupae	33	5.6	0.3
100-144 hr pupae	70	5.0	0.3

ANOVA

Source of variation	df	SS	MS	*F*
Among ages	1	7.94[1]	7.94	1.40 (N.S.)
Within ages	101	571.62	5.65	
Total	102	579.56		

F at 0.5% (1,100) = 3.94

[a] Animals arrested at various times over the 2-day interval are pooled.

[b] At least 1000 cells were scored for each animal.

The Gap between Thymidine Incorporation and Mitosis Is Over 20 Hours in the TAGs

For quantitative analyses of the parameters of the cell cycle, we chose to concentrate on the TAGs. By using autoradiography to detect incorporation of [^{3}H]thymidine, we found that precursors were readily incorporated into nuclear DNA from the beginning of the pupal stage through Day 5. We concentrated our further analyses on times of high mitotic activity, namely Days 1-2 and Days 4-5. The mitotic indices were not significantly different between these two periods (Table 1) but, as shown in Table 2, the mean labeling indices at 4-5 days were significantly higher than those at 1 day.

After injection of [^{3}H]thymidine, we sampled the developing glands at intervals of 4-6 hr for the following 60-70 hr. There was no increase in the percentage of labeled nuclei after the first 4 hr (Fig. 3). Thus, labeling patterns indicated that most of the [^{3}H]thymidine incorporation took place in the first 4 hr. The fractions of the labeled mitotic figures (FLM) varied widely, from below 1% to over 70%. The data are shown in Fig. 4A for animals injected at 1 day and in Fig. 4B for animals injected at 4 or 5 days.

Very few labeled mitoses were seen in the first 16 hr after administration of the label. For animals injected at 1 day of pupal age, the FLM began to rise at 16 hr and to increase dramatically at about 24 hr after administration of the label, to reach its peak about 10 hr later, and then to decline (Fig. 4A). For

TABLE 2
LABELING INDICES OF TAGs

Age at injection[a]	Number	Labeling indices Mean ± SE (%)
24-hr pupae	50	26.6 ± 0.7
96-hr pupae	47	30.1 ± 1.0
120-hr pupae	32	38.9 ± 1.2

ANOVA

Source of variation	df	SS	MS	F
Among ages	2	3037.0	1518.5	39.18***[b]
Within age	126	4883.5	38.76	
Total	128	7920.5		

$F_{0.001\,(2,126)} = 7.30$

[a] Ages were = ±2 hr. at least 1000 cells were scored for each animal.
[b] Very highly significant difference.

pupae injected on the fourth or the fifth day, the FLM began to accelerate somewhat earlier (before 20 hr), to reach its peak earlier (30 hr), and to decline earlier than in the first mitotic bout (Fig. 4B).

The G_2 Phase Is Longer Than the G_1 Phase of the Cell Cycle in the Pupal TAGs

Microspectrophotometric measurements of absorbance of Feulgen-stained nuclei and cytometric measurements of fluorescence from propidium iodide-stained nuclei provided two independent sets of data which are in general agreement. Both the frequency histogram (Feulgen, Fig. 5) and the Quickestimate calculations (propidium iodide, Table 3) show that 2C cells predominate just after pupal ecdysis when mitotic activity is low and that almost all of the cells arrest with 4C DNA in the late pupa. By comparison of the peak fluorescence from *Drosophila* and chicken nuclei with that from TAG nuclei, we estimate that the DNA content of 2C nuclei of *T. molitor* is about 2 pg (Table 4).

For the mitotic peak times (1 and 4 days), we analyzed the distributions in more detail. The proportion of cells in the S phase and a better estimate of the proportion in G_1 and G_2 + M were obtained by using iterative computer modelling (Gray and Dean, 1980; see Materials and Methods). The raw data and the corresponding model distributions are shown in Fig. 6.

The *duration* of each phase of the cell cycle was calculated. We combined the data on nuclear DNA content with that from the FLM experiments to solve several simultaneous equations which have been derived on the assumption of exponential growth in the cell population (Cleaver, 1967). The results of these calculations at 4 days are shown in Fig. 7. The entire cycle is 48 hr long; G_1, S, G_2, and M last 13, 14, 17, and 4 hr, respectively. As will be discussed below, we believe that the assumption of asychronous exponential growth is not correct for the 1-day organs.

DISCUSSION

Cell Cycles in the Accessory Glands of Mealworm Pupae

In the present study, we reported increases in cell numbers and variations in mitotic indices during pupal development of male accessory glands. The cell numbers in the BAG increase 3-fold, suggesting somewhat less than two rounds of cell division in the pupa, while in

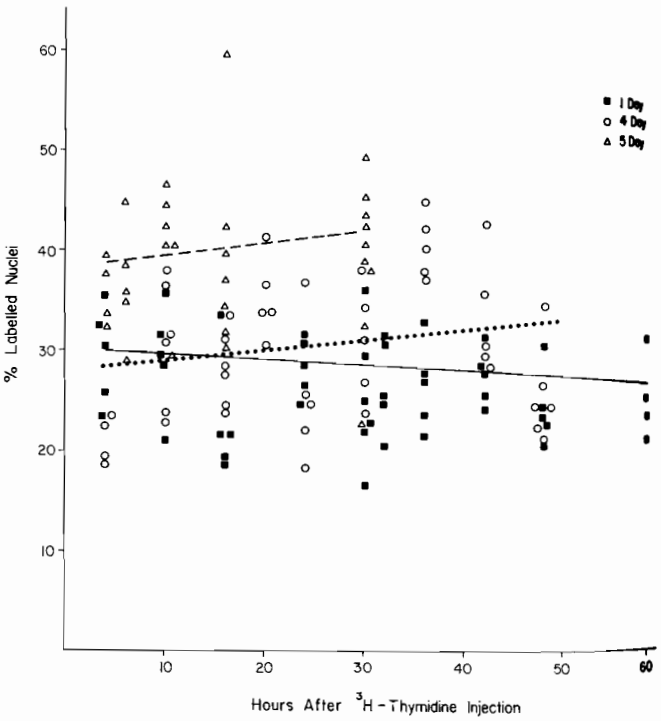

FIG. 3. Regression of labeling index versus hours after injection of [^{3}H]thymidine into the pupal TAG. Age at injection of label was 1 (solid line), 4 (dotted line), and 5 days (dashed line). One thousand cells were counted in each animal. Labeling index included both interphase and mitotic cells. The regression equations are as follows:

Age at injection	Equation	Correlation coefficient (r)
1 day	$y = -0.067x + 30.7$	−0.178
4 days	$y = 0.108x + 28.3$	0.209
5 days	$y = 0.216x + 36.4$	0.332

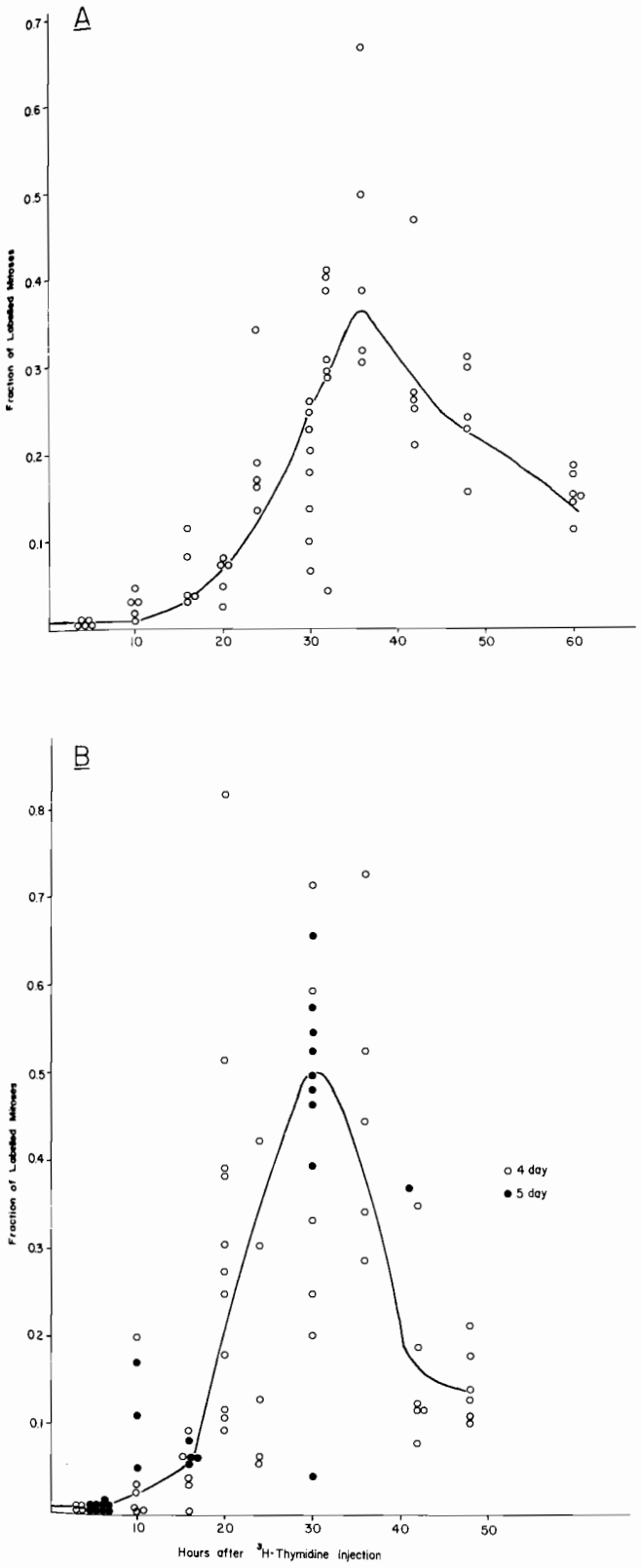

FIG. 4. Fraction of labeled mitoses in TAGs which were labeled with [³H]thymidine at 1 day (A) or at 4 and 5 days (B) of pupal age. Each datum point is based on 1000 cells counted in a different experimental animal.

TABLE 3
FRACTION OF TAG CELLS IN PHASES OF THE CELL CYCLE AS DETERMINED BY FLOW CYTOMETRY[a]

Organ	Age of tissue	Fraction of cells in G_1	Fraction of cells in G_2 + M
TAG & BAG	0-hr pupa	0.55	0.28
TAG	1-day pupa	0.46	0.28
TAG	2-day pupa	0.33	0.43
TAG	3-day pupa	0.40	0.43
TAG	4-day pupa	0.39	0.46
TAG	5-day pupa	0.31	0.44
TAG	6-day pupa	0.13	0.60
TAG	7-day pupa	0.10	0.70
TAG	6-day adult	0.06	0.93

[a] Nuclei were stained with propidium iodide. Proportion of cells in G_1 and G_2 + M were estimated by the Quickestimate method as described under Materials and Methods.

the TAG cell numbers increase 14-fold, suggesting almost four rounds of mitosis.

We have used cell population growth, fractions of labeled mitoses, cytometry, and cytophotometry to investigate the parameters of the cell cycle in the developing TAG (Fig. 6). Results from the three techniques are quite consistent. The FLM analysis indicated a G_2 duration of slightly less than 20 hr; the cytometric and cytophotometric results support this conclusion of a relatively long G_2. The calculated values for S-phase cells at 1 and 4 days are 26.9 and 27.5% (Fig. 6) are in good agreement with the labeling indices of 26.5 and 30.1% (Table 2). The calculated length of one cell cycle at 4 days is 44 hr (Fig. 6), a result that is in close agreement with the estimate of doubling time (46 hr) which came from the rate of increase of the cell population (Fig. 1). At 1 day of pupal age, we believe that the standard equations (from Cleaver, 1967; given in the legend for Fig. 7) are inappropriate to model

TABLE 4
DNA CONTENT OF TAG CELLS OF *Tenebrio molitor*

Cell type	DNA class	Peak fluorescence signal[a]	Estimated DNA content/ nucleus (pg)[b]
High-gain setting			
D. melanogaster brain	2C	170	0.40
T. molitor TAG	2C	900	1.9
Low-gain setting			
T. molitor TAG	2C	163	2.2
Chicken RBC	2C	185	2.5

[a] Propidium iodide stain.

[b] Assuming 0.40 pg DNA per 2C nucleus of *D. melanogaster* and 2.5 pg DNA per nucleus of chicken erythrocytes.

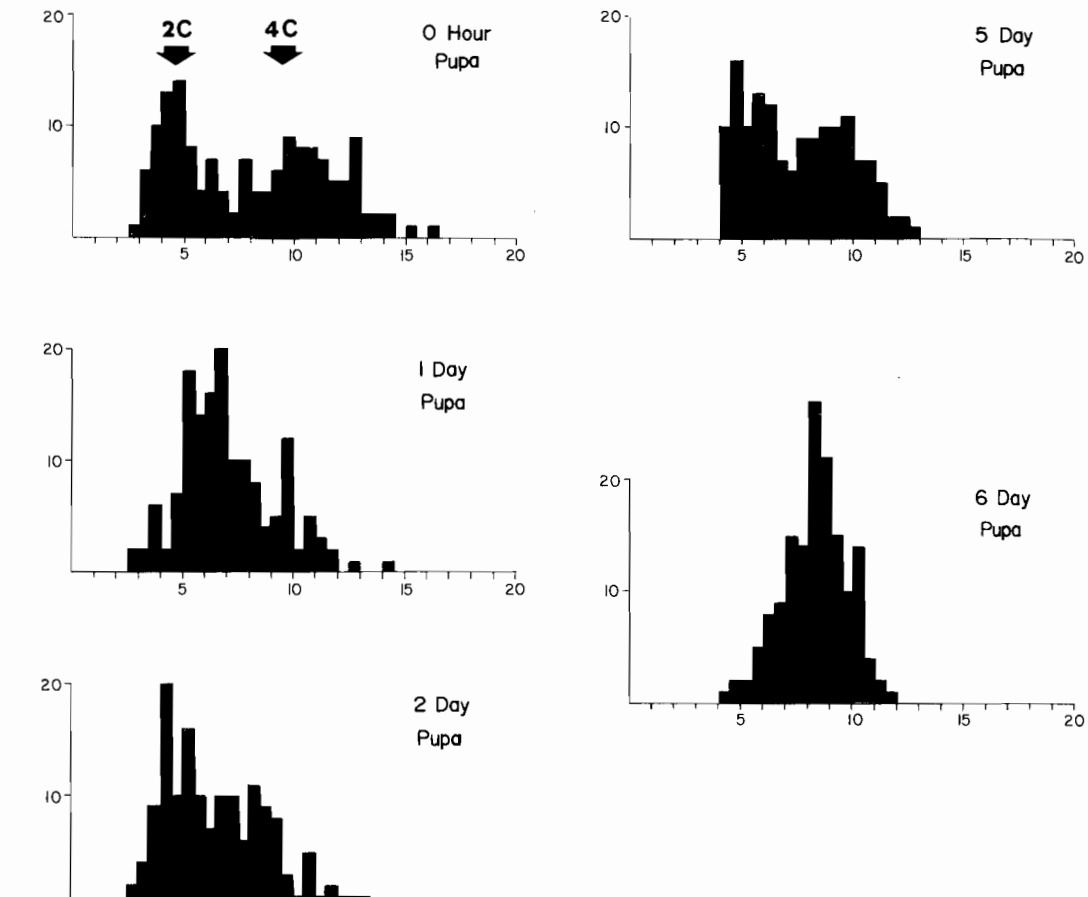

FIG. 5. The distribution of relative absorbance values of Feulgen-stained nuclei within TAGs during the days of the pupal stage and in the young adult. At least 150 nuclei were measured at each age. Absorbance values are given in machine units.

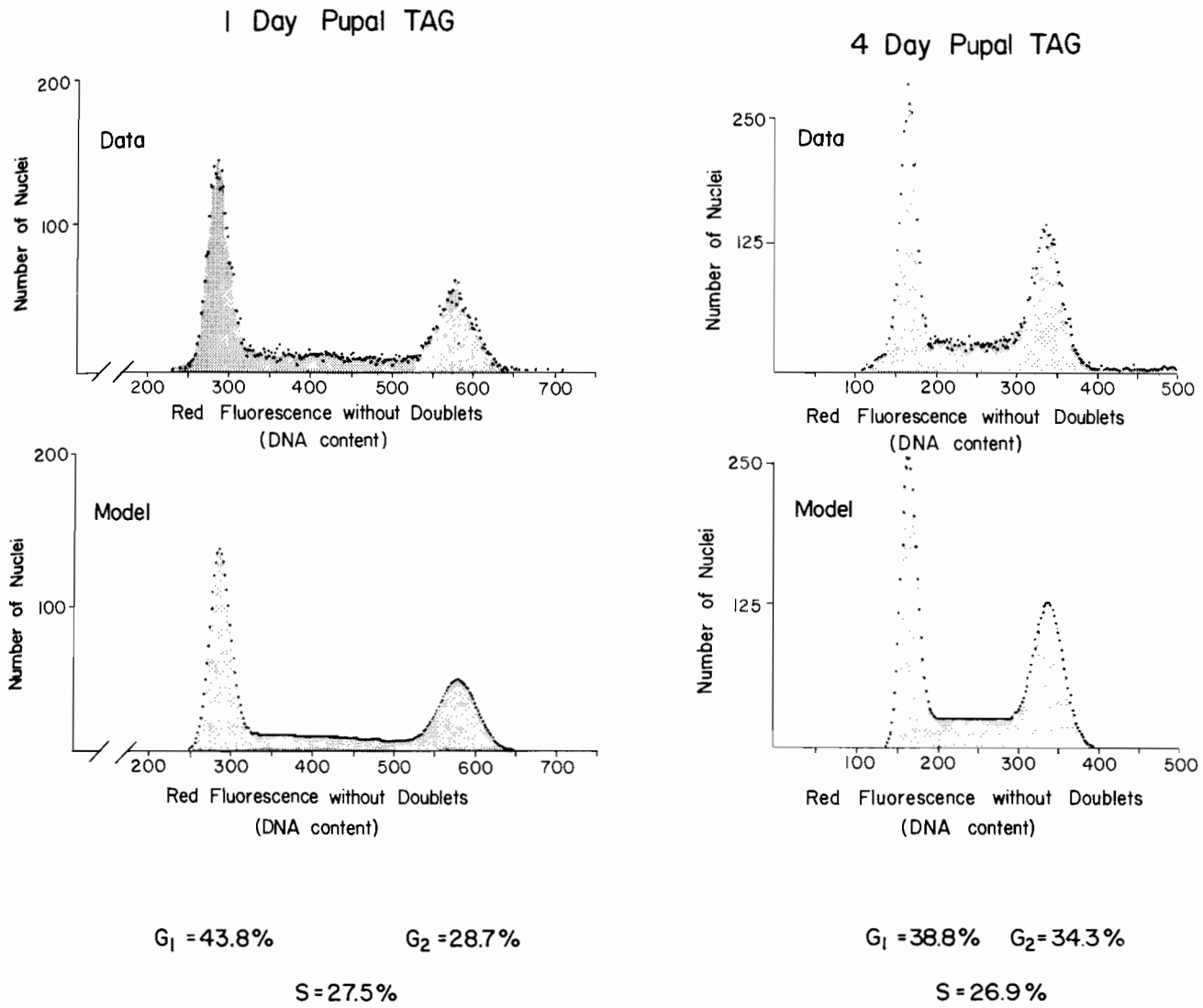

FIG. 6. The comparison of the experimental data and the model population for cells of the TAG at 1 and 4 days of the pupal stage. The root mean square error for 1 day is 0.69 and for 4 days is 1.33.

the cell population. These equations apply only when cell populations are asychronous and growing exponentially. But at 1 day after ecdysis to the pupa, the mitotic rate has just accelerated and the 2C:4C ratio has not yet stabilized.

When we measure t_2 (duration of G_2 + 1/2M) at 50% peak height on the FLM curves (Cleaver, 1967), the value is 22 hr for 1-day animals (Fig. 4A) and 19 hr for 4-day animals (Fig. 4B). These data might be taken as an indication that the minimum length of G_2 of the TAG cells is greater at 1 day than at 4 days. However, we believe that an alternative explanation is more likely. A careful comparison of the two curves (Fig. 4) reveals an interesting similarity and three further differences. The similarity is the inflection point: the first labeled mitoses appear at slightly over 16 hr in both ages, suggesting that the minimum length of G_2 is unchanged between 1 and 4 days. The three differences are in slope, in peak height, and in the time of the FLM peak. In 1-day animals, the slope is more gradual, the peak is lower, and its maximum value is reached 10 hr later than in 4-day animals. Since our label appears to be incorporated as a pulse, the first rise of the curve is independent of shifts in the proportion of cells in the growth fraction and of variations in the length of G_1. We believe that the broader, later peak at 1 day reflects greater heterogeneity in the length of the G_2 phase at 1 day than at 4 days.

The Functions of G_2 Arrested Cells

In some tissues, G_2-arrested cells constitute a sort of "ready reserve" which can be rapidly recruited for

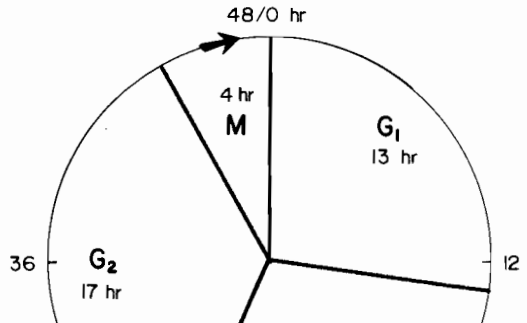

FIG. 7. The cell cycle at 4 pupal days in the TAG. Calculations were made using formulas from Cleaver (1967). t_2 (the duration of G_2 + 1/2 of mitosis) is the interval between thymidine injection and the point at which the FLM curves rise to 50% of peak height (from Fig. 4B, $t_2 = 19$ hr). T (the duration of one complete cycle) was calculated using the following formula. The fraction of cells in G_2 was estimated by assuming that 1/2 of the mitotic cells (shown in the mitotic index) are in prophases and subtracting this prophase value (2.5%) from the G_2 + prophase result derived from the polynominal iterative model (Fig. 6). T, the duration of one complete cycle, was calculated by fraction of cells in $G_2 = \exp t_2 \ln 2/T - 1$. t_m (the duration of mitosis) was calculated using the mitotic indices (Table 1) and the following formula: $t_m = (MI)(T)/\ln 2$. t_s (the duration of the S phase) was calculated using the labelling indices (Table 2) and the following formula: L.I. = $\{\exp[(t_s \ln 2)/T] - 1\} \cdot \exp(t_2 \ln 2)/I$. t_1 (the duration of the G_1 + 1/2 of mitosis) was calculated at 14 hr by the following formula. The fraction of cells in G_1 is from our Fig 6. Fraction of cells in $G_1 = 2\{1 - \exp[(-t_1 \ln 2)/T]\}$. t_{G_1} (duration of G_1) = $t_1 - 1/2\ t_m$; t_{G_2} (duration of G_2) = $t_2 - 1/2\ t_m$.

production of more cells. In the ear epidermis of the mouse, the G_2-arrested cells, which comprise but a small fraction of the population, are the first to undergo mitosis in response to wounding (Koburg and Maurer, 1962; Gelfant, 1963). An analagous arrest occurs after starvation in multicellular and unicellular plants. In carbohydrate-starved cultures of the primary root meristems of some legumes (Van't Hof, 1966; Van't Hof and Kovacs, 1972; Webster and Van't Hof, 1970), about half the cells arrest in G_2 and the other half in G_1. When sucrose is added to the cultures, both arrested populations proceed onward through the cycle. A G_2 arrest occurs in cultures of *Tetrahymena pyriformis* which have been grown to high densities and then starved (Cameron and Bols, 1975). Both G_1 and G_2 arrest may occur in the same cell type. Examples include the root meristem discussed above (Van't Hof *et al.*, 1973) and the K_c cell line of *Drosophila*. In the latter case, the cells arrest in G_1 when grown to high densities (akin to starvation) but arrest in G_2 when treated with ecdysterone (Stevens *et al.*, 1980).

Although terminal differentiation has been most commonly associated with cells in G_1 (Pardee *et al.*, 1978), some fully differentiated cell types are characteristically tetraploid or polyploid. During flower development in clone 4430 of *Tradescantia*, the anthers arrest in G_2 just before terminal differentiation (Kudirka and Van't Hof, 1980). In the epidermis of pupal *Galleria mellonella* (Wolbert and Kubbies, 1983) and *T. molitor* (Besson-Lavoignet and Delachambre, 1981) and in the imaginal discs of dipterans (Van der Want and Spreij, 1976; Egberts, 1979; Fain and Stevens, 1982; Graves and Schubiger, 1982), the majority of the cells have 4C DNA content and thus are blocked in G_2. Polyploid cells are common in secretory tissues including insect fat body (Locke, 1980), mammalian liver (Bresnick, 1971), silk glands of Lepidoptera (Suzuki, 1977), and polytene salivary glands of Diptera. In tissues of many insect orders, the tetraploid cells go on to divide at regular intervals (Wigglesworth, 1973; Romer and Eisenbeis, 1983) and the ploidy of some cells increases with each succeeding larval molt. At metamorphosis, the polyploid cells either undergo degeneration (as is characteristic of Hymenoptera, Lepidoptera, and Diptera) or survive to the adult (as in Coleoptera) (references in Romer and Eisenbeis, 1983). In *T. molitor*, a large fraction of the cells of the sternal epidermis (Besson-Lavoignet and Delachambre, 1981), the TAG (this paper), and the BAG (unpublished data from this laboratory) become tetraploid before beginning to secrete their cell-specific products.

Regulation of the G_2 Phase of Cell Cycles in Insects

Ecdysteroid peaks have been linked to G_2 arrests in several different insect cells. Cells of the K_c line of *D. melanogaster* change in morphology, switch their pattern of protein synthesis, and arrest the cycle in G_2 in response to ecdysterone (Stevens *et al.*, 1980; Courgeon, 1982; O'Connor *et al.*, 1980; Cherbas *et al.*, 1980; Sarakis *et al.*, 1980). After ecdysteroid titers rise at the end of the third instar of *D. melanogaster*, many of the cells in the imaginal leg discs appear to be arrested in G_2 (Graves and Schubiger, 1982). In the sternal epidermis of *T. molitor*, G_2 arrest is correlated with increased ecdysteroid titers in the last larval and pupal instars (Besson-Lavoignet and Delachambre, 1981).

Cell cycles in different tissues of the same insect are often out of phase with one another (e.g., Kato and Oba, 1977; Romer and Eisenbeis, 1983). The same pupal ecdysteroid peak that coincides with arrest in the sternal epidermis (Besson-Lavoignet and Delachambre, 1981) is also correlated with continuing a mitotic

activity and probably with a greater uniformity in the length of G_2 in the TAGs and the BAGs (this paper, Happ and Happ, 1982; Grimes and Happ, 1980). As we demonstrate in a companion paper (Szopa *et al.*, 1985), ecdysterone accelerates cell division in the TAGs and the BAGs during culture *in vitro*. The importance of this pupal ecdysterone peak to the terminal differentiation which follows remains to be investigated.

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