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# A reappraisal of the hormonal regulation of larval fat body histolysis in female *Drosophila melanogaster*

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*Abstract.* The histolysis of larval fat body cells in adult female *Drosophila melanogaster* was examined in wild type and mutant animals. The fat body cells of wild type (Canton-S), *apterous*$^{56f}$ homozygotes, *apterous*$^{78jts}$ homozygotes and heterozygotes, *apterous*$^{4}$/+, *ecdysoneless*$^{1}$ homozygotes and heterozygotes all underwent histolysis normally during the 72 h following adult eclosion. Only in the case of *ap*$^{4}$/*ap*$^{4}$ adults did the cells fail to histolyze normally. The fat body cells of both diapausing and non-diapausing wild type females underwent histolysis at the same rate. Attempts to demonstrate histolysis in vitro were unsuccessful, even in the presence of juvenile hormones (JHs), larval ring glands, or adult ovaries. In all strains other than the *ap*$^{4}$ homozygotes, a significant proportion of larval fat body cells were dead at any time while the *ap*$^{4}$/*ap*$^{4}$ animals, almost all cells remained viable. It is postulated that fat body cell lysis following eclosion is not a JH-mediated event, but is elicited by an as yet unidentified factor(s), possibly originating in the ovary.
*Key words.* Cell death; juvenile hormone; endocrine mutants; diapause.

Following pupation, the larval fat body of *Drosophila melanogaster* undergoes disaggregation into separate cells[1,2], and subsequent histolysis. By day 3 of adult life, no intact cells are present. Day[3] showed that the removal of the corpus allatum from young *Sarcophaga* adults resulted in the retention of larval fat body tissues, and subsequently, the role of intrinsic factors such as juvenile hormones (JHs) in *Drosophila* fat body histolysis has been examined[4–6]. The resulting data indicated that larval fat body cells undergo a preprogrammed cell death after eclosion which is apparently under endocrine control. Convention has referred to the disappearance of these fat body cells as histolysis, but since the cells are disaggregated prior to lysis, perhaps cytolysis might be a more accurate term.

The comparison of wild type and mutant Drosophila (*apterous*$^{4}$, a non-vitellogenic, JH-deficient mutant which dies precociously) revealed that the fat body of the mutants failed to undergo complete histolysis[5]. Partial histolysis to about 20% of the level typical of newly eclosed wild type adults did occur, and transplantation experiments showed that this lack of complete histolysis was not due to a defect in the fat body cells, but rather to the internal environment of the mutant flies[4,5]. Further studies showed that the abdominal fat body of head-ligated wild type adults simulated that of *ap*$^{4}$ homozygotes, and that the application of a JH mimic resulted in normal histolysis and vitellogenesis in both the mutant animals and isolated abdomens, suggesting strongly that JH is involved in these processes[6], but not proving it unequivocally.

In the present report, we reinvestigated the phenomenon of fat body histolysis using other alleles of the *apterous* gene, *ap*$^{56f}$ and *ap*$^{78jts}$. Corpora allata from *ap*$^{56f}$ female adults produce the same low levels of JHs as *ap*$^{4}$ animals when compared to wild type flies[7,8] yet these mutants are completely vitellogenic and fertile, and live far longer than *ap*$^{4}$ animals. Corpora allata from temperature sensitive *ap*$^{78jts}$ homozygous adults[9] reared at the restrictive temperature produce amounts of JH intermediate between the wild type and severe *apterous* levels (D. Segal, personal communication). In the present study, we use these mutants to determine whether low JH production is always associated with a failure of normal fat body histolysis. A second vehicle for this examination is the non-vitellogenic, adult female reproductive diapause of *Drosophila melanogaster*[10] which is associated with low levels of JH production by the isolated corpus allatum in vitro[11]. The possibility that ecdysteroids may be involved in these processes was addressed by using an ecdysteroid deficient mutation, *ecdysoneless*$^{1}$ [12].

## *Materials and methods*

*Animals.* Wild type *Drosophila melanogaster* (Canton-S strain) and all mutant strains were maintained under an LD12:12 photoperiod at 25 °C unless otherwise indicated. Diapausing animals were produced by having wild type animals eclose to the adult stage under an LD12:12 photoperiod at 12 °C[10] while non-diapause flies eclosed under a photoperiod of LD18:6 at 12 °C. Under these conditions, ovaries were vitellogenic about

ten days after adult eclosion in the non-diapause animals. All of the mutant strains used in this study, other than the *ap*$^{56f}$ animals, were maintained as balanced stocks in which roughly one third of the population were homozygous for the particular mutation. The *ap*$^{56f}$ animals were maintained as a homozygous stock. The temperature sensitive *ap*$^{78jts}$/*In(2LR)Gla, GlaBcElp* (second chromosome balancer) strain was reared at 18 °C and upshifted to 25 °C or 27 °C (both restrictive temperatures) within minutes of eclosion, or at intervals prior to the estimated time of eclosion. Although it was thought that JH-deficient phenotypes require upshift within 24 h of pupariation[9], low levels of JH synthesis in vitro by isolated adult female corpora allata are noted if the upshift occurs immediately following eclosion (D. Segal, personal communication). The *y;ecd*$^{1}$/*TM3, Ser.y* + (third chromosome balancer) strain was reared at 18 °C and upshifted to 29 °C 24 h before adult eclosion. In each case, heterozygotes were generated with the corresponding balancer rather than with the wild type chromosome. All animals were reared on artificial *Drosophila* medium (Carolina Biological) supplemented with dried bakers yeast.

*Experimental procedures.* The progress of larval fat body histolysis in adult females following eclosion was examined by first mincing the flies with two pairs of fine forceps in MEM tissue culture medium (Lineberger Cancer Research Institute, University of North Carolina). The tissue was then sucked up into, and expelled repeatedly from, a Pasteur pipette to dissociate the clumps of fat body cells from the other tissues and to render them easier to count. Counting was done manually under a dissecting microscope according to established criteria[6]. Adult fat body cells were easy to distinguish from the larval cells before histolysis by their formation into large translucent sheet-like structures, whereas the larval cells remained separate and more opaque in appearance.

The viability of fat body cells was assessed by trypan blue exclusion. The dye was added at a concentration of 0.1% to cells in MEM, and after a few min at room temperature examined under a dissecting microscope. Cells which excluded the dye were considered viable while those which failed to exclude the dye stained blue and were considered dead. It should be noted that living cells with their basement membranes partially digested by dilute solutions of collagenase may also fail to exclude the dye (F. M. Butterworth, personal communication). For the purpose of this study however, those cells failing to exclude the dye were considered dead.

An attempt was made to repeat the observation that the application of the JH analog methoprene (Sandoz) to the abdomens of newly emerged *ap*$^{4}$ homozygous females caused a partial recovery of the histolytic process[6]. Methoprene was dissolved in acetone to a concentration of 0.17 mg · ml$^{-1}$ and a volume of 0.5 µl (0.085 µg) was applied topically to the ventral surface of the abdomen. The extent of histolysis was examined after a 24-h incubation at 25 °C.

*In vitro studies on the rate of fat body histolysis.* Adult females (Canton-S) were collected 4 h after eclosion and their abdomens opened under MEM. Tissue debris was removed and the fat body cells were washed with fresh MEM and finally resuspended in MEM supplemented with 25 µg · ml$^{-1}$ ampicillin (US Biochemicals). Six 20-µl aliquots of this cell suspension, containing approximately 200 cells each, were placed carefully in the bottom of a 24-well microtitre plate (Falcon 3045). The number of cells in each droplet was counted at time zero, 18 h and 42 h. Six aliquots of the cell suspension were co-incubated with third instar larval ring glands dissected clear of surrounding tissues. A further six aliquots were co-incubated with pairs of ovaries isolated from females 12 h after eclosion. All incubations were carried out at 25 °C.

Another set of experiments tested the effects of $JHB_3$, JH III and methyl farnesoate, juvenoids produced by *Drosophila* corpora allata in vitro[7,13], on the histolysis of fat body cells in vitro. Canton-S females were collected 4 h after eclosion and their fat body cells treated as above. Aliquots of cells (10 µl) were placed in 10 µl of MEM supplemented with the juvenoid to be tested to yield a final concentration of 0.1 mM. In addition, the JH esterase inhibitor OTFP[14] (a gift of M. Roe: final concentration 0.5 µM) was added to the MEM immediately prior to the incubations. The number of cells in each droplet was counted at time zero, 19 h and 48 h after the incubation was initiated. The viability of the fat body cells in these incubations was assessed by trypan blue exclusion as discussed above.

## *Results*

*Larval fat body histolysis.* Figure 1A shows the rate of histolysis of larval fat body cells in Canton-S adult females following eclosion at 25 °C. By 72 h after eclosion, almost all of these cells had lysed, and of those remaining, 55% were dead. Indeed, the cell death profile of these cells as determined by trypan blue exclusion reveals a significant number of dead cells that have not yet lysed at any given time point. A similar profile of lysis and cell death was noted for the fat body of male wild type animals (data not shown) and females homozygous for the *ap*$^{56f}$ mutation (fig. 1B), a JH deficient mutant[8]. In the temperature sensitive *ap*$^{78jts}$ homozygotes (fig. 1C), the rate of fat body histolysis at the restrictive temperature (25 °C) is the same as for the fat body of *ap*$^{78jts}$/*In(2LR)Gla,GlaBcElp* heterozygotes which contain one wild type copy of the *apterous* gene. The rate is essentially the same as observed for the fat body of wild-type and non-conditional *ap*$^{56f}$ homozygotes (figs 1A, B). The *ap*$^{78jts}$ allele is also believed to be JH deficient under these conditions i.e. upshift

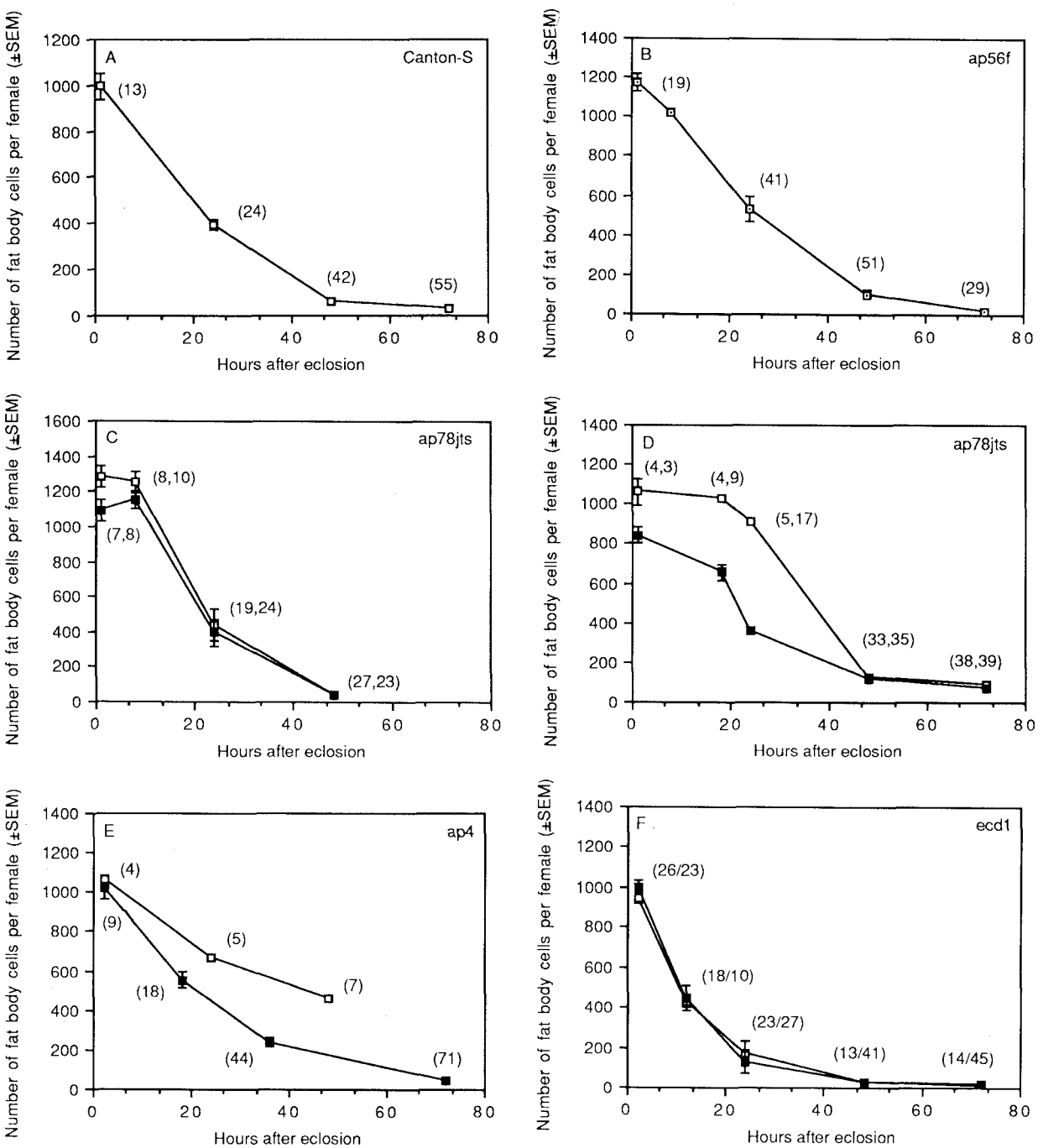


Figure 1. The number of larval fat body cells present in adult female *Drosophila melanogaster* following eclosion. *A* Canton-S at 25 °C; *B* $ap^{56f}$ homozygotes at 25 °C; *C* $ap^{78jts}$ homozygotes (open squares) and $ap^{78jts}$ heterozygotes at 25 °C (filled squares); *D* $ap^{78jts}$ homozygotes (open squares) and $ap^{78jts}$ heterozygotes (filled squares) upshifted from 18 °C to 27 °C 96 h prior to eclosion; *E* $ap^{4}$ homozygotes (open squares) and $ap^{4}$ heterozygotes at 25 °C (filled squares); *F* $ecd^{1}$ homozygotes (open squares) and $ecd^{1}$ heterozygotes (filled squares), both at 29 °C. The numbers in parentheses represent the percentage of cells from each stage which have not yet lysed but fail to exclude trypan blue dye. In C, D and F, the first number represents the homozygotes. Each point is the mean ± SEM of 5 determinations.

from 18 °C to 25 °C immediately following eclosion (D. Segal, personal communication). When these animals were upshifted to 27 °C, an even more restrictive temperature for JH production, there was still no difference between fat body cytolysis for the homozygotes and heterozygotes (data not shown). To determine the limits of the temperature sensitive period for fat body histolysis, as opposed to the period for JH production, animals were upshifted at earlier and earlier times prior to eclosion, determined by the times of pupariation. The profiles of histolysis for females upshifted to 27 °C 96 h prior to eclosion are shown in figure 1D. While there

seems to be a delay in the initiation of lysis, the end point is the same for the homozygotes and the heterozygotes, with similar levels of dead cells in the fat body population of each. Even at 120 h prior to eclosion, there does not seem to be an effect on the process of histolysis (data not shown), and at each time point, a similar profile was noted with the fat body of male flies (data not shown).

Only in the homozygous $ap^4$ females does the pattern of fat body histolysis deviate from the above (fig. 1E). The fat body of these animals is not lysed completely and the flies die within 48 h of eclosion, a time when about 50% of the cells are unlysed in contrast to the heterozygotes in which cytolysis has occurred in about 80% of the cells. Similar results were seen with males homozygous for the $ap^4$ mutation (data not shown). In the Canton-S, $ap^{56}$ and $ap^{78jts}$ female adults, the number of cells remaining which failed to exclude trypan blue dye and therefore were considered to be dead varied at any particular time point but was generally above 25%. The level of mortality among unlysed cells in the $ap^4$ homozygotes was much lower, i.e. a maximum of 7%. The control fat body of heterozygous $ap^4$ animals exhibited profiles of normal cell death and histolysis.

*Possible endocrine control of fat body histolysis.* When the effect of methoprene on the process of histolysis in newly eclosed $ap^4$ homozygous females was analysed, it was clear that we failed to confirm previous results[6]. Females assayed 24 h following the application of 85 ng of methoprene contained $785 \pm 29$ (SEM) fat body cells, 4% of which were dead ($n = 16$). Females to which acetone alone was applied contained $872 \pm 43$ (SEM) fat body cells of which 3% were dead ($n = 9$). An unpaired t-test comparing these data gave $p = 0.118$ ($d_f = 23$), showing no significant difference between these treatments.

At the restrictive temperature of 29 °C fat body histolysis in females homozygous for the ecdysteroid deficient temperature sensitive mutation $ecd^1$ was no different than that of fat body from heterozygotes used as the control (fig. 1F). Both rates of histolysis were higher than those observed for the Canton-S or *apterous* mutants at 25 °C but this was possibly due to the higher temperature. However, the proportion of dead fat body cells in the $ecd^1$ mutant was similar to that of wild type flies.

When wild type *D. melanogaster* females eclose under a short-day photoperiod (LD12:12) at 12 °C, they enter a state of previtellogenic, reproductive diapause[10], in which the in vitro production of JH by the corpus allatum is reduced to nearly the lower limits of detection[11]. However, in contrast to the $ap^4/ap^4$ mutants which show similar low levels of in vitro JH production[7], diapausing females survive for many weeks and regain the ability to deposit yolk and develop normal eggs upon exposure to higher temperatures, transfer to a long-day photoperiod, or after the application of JH bisepoxide ($JHB_3$). Homozygous $ap^4$ mutant animals do not survive longer at 12 °C than they do at 25 °C, indicating that the lower temperature experienced by diapausing females does not compensate for the lower levels of JH with regard to survival. The effect of the diapause state on fat body histolysis in wild type females was determined by examining lytic rates at 12 °C under both diapausing and non-diapausing inducing photoperiods. Figure 2 shows that larval fat body cells from flies representing both physiological conditions are lysed at about the same rate. At this low temperature, vitellogenesis was slow in the non-diapausing flies but did occur at a rate significantly greater than that in diapausing animals. Vitellogenic females were first seen after 8 days under non-diapause inducing conditions, and by day 10 the ovarioles of all flies were at the vitellogenic stage 9/10[15]. Very few vitellogenic females were present under diapausing conditions, and those that did show even the first signs of yolk deposition were removed from the sample[10].

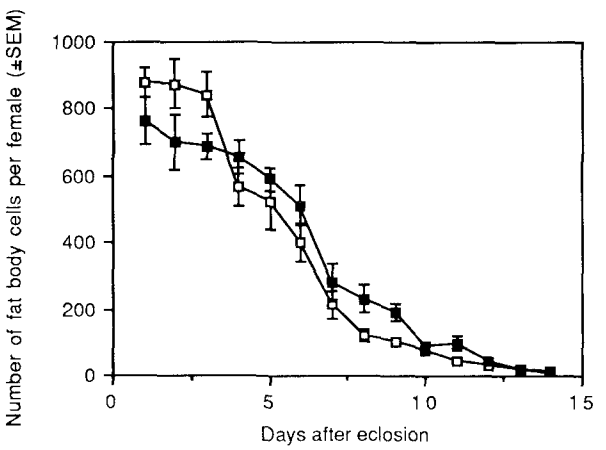


Figure 2. The number of larval fat body cells present in adult female *Drosophila melanogaster* (reared in 12L:12D at 25 °C) following eclosion at 12 °C into a diapause inducing photoperiod (12L:12D) (filled squares) or a non-diapause inducing photoperiod (18L:6D) (open squares). Each point represents the mean ± SEM of 5 determinations.

Attempts to establish conditions suitable for the induction of female fat body histolysis in vitro were unsuccessful. Histolysis did not occur when wild type fat body cells isolated within 4 h of eclosion at 25 °C were incubated either with or without third instar post-feeding larval ring glands (data not shown). The cell number remained constant and there was little change in the gross appearance of the cells during the experiment. Similar results were obtained when cells were co-incubated with pairs of ovaries isolated from wild type females 12 h after eclosion. There was no significant effect of the JHs on the rate of histolysis in vitro (data not shown) and the JH esterase inhibitor OTFP alone had no effect.

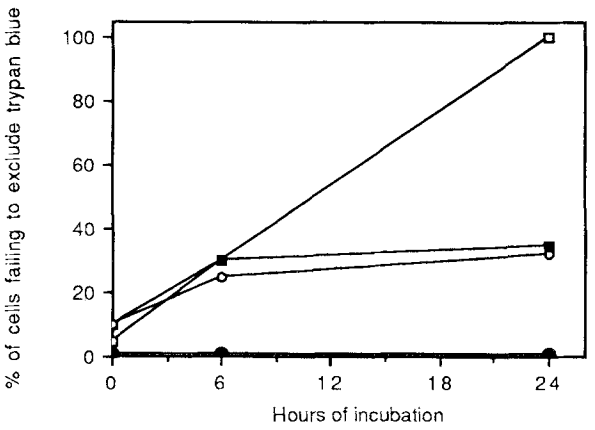


Figure 3. The percentage of larval fat body cells from *Drosophila melanogaster* which fail to exclude trypan blue dye after incubation in vitro at 25 °C. Filled circles, larval fat body isolated from post-feeding third instar larvae; open circles, fat body cells from pupae shortly after head eversion; Filled squares, fat body cells from pre-emergent adult females; open squares, fat body from adult females 4 h after eclosion.

When the viability of Canton-S female fat body cells isolated 4 h after eclosion at 25 °C was examined for trypan blue exclusion, it was noted that after a 24-h incubation at 25 °C, all cells stained blue, indicating that they were dead (fig. 3). Larval fat body cells isolated from third instar wandering larvae excluded the dye up to 48 h after incubation under the same conditions, indicating that they were viable. Fat body cells from pupae shortly after head eversion at 25 °C showed a steady increase in the number of dead cells, from 10% to 35% over 24 h in vitro. Similar results were seen with cells isolated from pre-emergent adults. These data indicate that cells isolated from females 4 h after eclosion have already undergone cell death programming at about the time of eclosion and that cell death may occur independently of histolysis.

*Discussion*

The role of JH in the regulation of larval fat body histolysis in *Drosophila melanogaster* has been studied previously using the $ap^4$ mutation[5,6]. Corpora allata from this JH deficient mutant produce very low levels of total JH ($JHB_3$, JH III and methyl farnesoate) in vitro[7,8]. A further indication of the JH deficient nature of this allele is that 0–1-day-old $ap^4$ homozygous adult females have 3–4 times less JH III per animal than do equivalent wild type animals[16]. No information on the whole body titer of $JHB_3$, the major product of the corpus allatum[13], is yet available so it is not possible to comment on the relevance of this JH to the regulation of post-eclosion development other than to note that it is more effective than JH III in breaking diapause[11], and is capable of eliciting vitellogenesis in *apterous* mutants following topical application (D. Segal, personal communication). It has been demonstrated that $ap^4/ap^4$ females failed to undergo complete larval fat body histolysis following eclosion, and that the animals died 2–3 days later[5,6]. The application of the JH mimic methoprene (ZR 515) to these animals caused near complete histolysis and the initiation of vitellogenesis[6]. From these data, it has long been inferred that JH is causally involved in the regulation of fat body histolysis and vitellogenesis. Although similar experiments showed that the application of methoprene could indeed elicit the initiation of vitellogenesis, there was no effect on histolysis (F. Butterworth, personal communication). We have repeated these experiments and were also unable to promote histolysis in these animals by the application of methoprene.

In the present study, we have confirmed the observations on the rates of histolysis in $ap^4$ homozygotes made previously[5,6] and extended them to demonstrate that normal histolysis occurs in those mutants which produce the same low levels of JH in vitro as $ap^4$ but that do not die precociously ($ap^{56f}$, and to a slightly lesser extent $ap^{78jts}$, under restrictive conditions). Furthermore, our data revealed that the rate of fat body histolysis is the same in diapause (non-vitellogenic) and non-diapause (vitellogenic) wild type flies, despite the fact that the levels of JH production in vitro are very low in the former (3.3 fmol/gland/h)[11]. These observations suggest that JH may not be the principle causal agent of fat body histolysis in adult female *D. melanogaster*, and that vitellogenesis and histolysis are not always linked.

If the low levels of JH produced in the $ap^{56f}$ and $ap^{78jts}$ mutants after eclosion[8] are sufficient to drive histolysis, one would expect a similar rate of histolysis in the fat body of $ap^4$ mutants. This does not occur, suggesting that: 1) differences in the sensitivity of the fat body cells of these alleles to the endogenous levels of JH may exist (perhaps as a result of differences in receptor amounts or affinities), 2) that JH is catabolized at a greater rate in $ap^4$ mutants, or 3) that JH is not involved. Since transplantation experiments with $ap^4$ homozygous fat body cells into wild type flies showed that the lack of histolysis in the mutants was not due to an intrinsic defect in the cells, but rather to an environment in which histolysis was not induced within the mutant adults[5], the first possibility seems unlikely. Wilson[17] stated that the examination of the rare $ap^4$ homozygote 'escapers' at 5–8 days after eclosion showed no larval fat body present. Presumably complete histolysis had occurred in these animals, even though levels of JH production in $ap^4$ homozygotes are very low[7], further suggesting that JH may not be involved. The data presented here for the rates of histolysis in the temperature sensitive $ap^{78jts}$ animals at the restrictive temperature show a great separation of the temperature sensitive period for JH production (upon eclosion)[8] and any effect on histolysis, which occurred almost normally

even when the animals were upshifted five days prior to eclosion. This suggests strongly that fat body histolysis is not regulated directly by JH release following eclosion.

If JH is not the controlling factor in the regulation of fat body histolysis as is suggested above, the identity of the principle modulator is conjectural. However, the observations of Butterworth and Bodenstein[4] who transplanted fat body cells from second instar larvae into adult males and females provide a clue. No histolysis was seen in normal males, partial histolysis occurred in females but not in ovariectomized females, and cells transplanted into males together with an adult ovary showed some histolysis. These observations suggest that a factor from the ovary may be responsible for histolysis. The possibility that this factor is an ecdysteroid is addressed here by the experiments with the ecdysteroid deficient homozygotes of *ecdysoneless*[1] which showed the same rate of fat body histolysis as occurred in the heterozygotes. The homozygotes at the restrictive temperature show low titers of ecdysteroids[12] (R. Mitchell, D.S.R. and L.I.G.,unpublished observations), low levels of ecdysteroid synthesis by isolated ovaries in vitro, and do not appear to develop vitellogenic ovaries (D.S.R. and L.I.G., unpublished observations). This suggests that an ecdysteroid is not the histolysis regulating factor at this stage of development, although isolated fat body cells from *Drosophila* embryos incubated with $4\ \mu g \cdot ml^{-1}$ ecdysone for 15 days in vitro lysed to a greater extent than those incubated in the absence of ecdysone[18], suggesting a role for ecdysone during early development. However, this concentration is considerably higher than the endogenous titre during embryonic and larval development (10–100 ng 20-hydroxyecdysone · g fresh weight $^{-1}$)[19]. In the Lepidoptera, 20-hydroxyecdysone may be involved in fat body cell autophagy[20]. The possibility still exists, however, that the failure of histolysis by fat body from $ap^4$ animals results from the failure of ovaries to produce a modulating factor other than an ecdysteroid. In the wild type adults, the internal environment which was initially restrictive, i.e., histolysis was induced, became more permissive by 4–8 days after eclosion[5]. These observations may be explained in terms of the fluctuations in the production of ovarian factors required for the completion of histolytic processes. The failure of the ovaries to cause histolysis in vitro may be due to the fact that they were isolated too late in adult development, i.e., 12 h after eclosion when production of these putative factors may have ceased, whereas Butterworth and Bodenstein[4] used ovaries from 'recently emerged females' which may have been early enough for the production of these factors to have an effect. Postlethwait and Jones[6] showed that de novo protein synthesis was required for fat body histolysis by injecting wild type animals with cycloheximide 4 h after eclosion and noting the persistence of fat body cells at 48 h. However, fat body cells already contain the enzymes required for histolysis when the fly ecloses[5,6]. They suggested therefore that the de novo protein may cause rupture of preexisting lysosomes and hence fat body histolysis, and that the synthesis of this protein is regulated by JH. The results reported in the present paper suggest that the synthesis of this protein factor can occur independently of the normal level of production of JH. Whether or not this protein is related to the ovarian factor discussed above remains to be determined.

Our failure to elicit histolysis in vitro raises some intriguing questions, especially when cell viability is considered. The in vitro conditions obviously did not cause the death of those cells not already programmed, since about 70% of cells from pre-emergent adults survived. However, all cells isolated from adults 4 h after eclosion died, indicating that the factor responsible for cell death differs from that regulating histolysis, and is released 0–4 h after eclosion, or very late in the pharate adult stage. It is therefore hypothesized that fat body histolysis may be a two-step process: the induction of cell death, and the subsequent lytic event. The failure of fat body from $ap^4$ animals to undergo normal histolysis may be the result of interference with the normal cell death program. The low number of dead cells present suggests that although cell death is reduced, those cells which do die are rapidly lysed, i.e., that the normal histolytic machinery is present but is unable to perform at the same rate as in wild type flies.

The fact remains that JH production in vitro by the corpora allata from $ap^{56f}$ and $ap^{78jts}$ mutant animals (under restictive conditions) is lower than by glands from wild-type animals[8] (D. Segal, personal communication). Despite this, processes thought to be JH dependent are able to continue as well in $ap^{56f}$ as in the wild type, with the exception of normal reproductive behavior[21,22]. The *apterous* gene is considered a complex locus with pleiotropic functions and it is possible therefore to separate certain functions of this gene, e.g., winglessness, reproductive capacity and precocious adult death[17]. Both $ap^4$ and $ap^{56f}$ homozygotes are completely wingless and show low levels of JH production in vitro, yet $ap^{56f}$ survives normally. Other alleles, e.g. $ap^c$, and $ap^c$ are wingless yet show normal levels of JH production during both larval and adult development (D.S.R. and L.I.G., unpublished data, and D. Segal, personal communication). Thus the simple lack of JH is not sufficient to account for all the effects exhibited by $ap^4$, and may not be directly relevant to the process of histolysis. Recently an *apterous* cDNA has been sequenced that encodes a member of the LIM family of regulatory proteins involved in the transcriptional regulation of developmentally regulated genes[23]. This gene appears therefore to be capable of eliciting a number of independent developmental processes which are not

necessarily dependent upon normal JH production, one of which may be the induction of larval fat body death and subsequent lysis.

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