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Regulation of Synthesis of Larval Serum Proteins after Transplantation of Larval Fat Body into Adult *Drosophila melanogaster*

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The larval serum proteins, LSP1 and LSP2, of *Drosophila melanogaster* are synthesized by the fat body during the third instar. We examined the potential for LSP synthesis by fat body implants in adult flies. Fat body from third instar donors will continue to synthesize LSPs in both males and females. Implants from late second instar larvae will start synthesizing LSP1 and LSP2 in females but only LSP1 in males, suggesting that regulation of these proteins is not the same and that the physiological milieu in the two sexes differs. The newly synthesized LSPs are secreted into the hemolymph for approximately 48 hr when secretion stops but synthesis continues. This sequence follows the pattern for LSP secretion *in situ*. Fat body from mid second instar larvae is variable in its ability to synthesize LSPs. LSPs are not detected in implants from first instar larvae despite there being a high level of protein synthesis in the implant and considerable growth of the fat body cells. We conclude that there is a critical stage of differentiation during the latter half of the second instar when the fat body becomes independent of the larval milieu and can synthesize LSPs in the adult. © 1986 Academic Press, Inc.

INTRODUCTION

The major proteins synthesized in *Drosophila* larvae are the larval serum proteins (LSPs). They are synthesized only by the fat body and only during the third instar (Sato *et al.*, 1982). LSP1 consists of three subunits (α, β, γ) and LSP2 a single subunit. The LSP1α gene is at 11A on the X chromosome, the LSP1β gene at 21D,E on chromosome 2L, and the LSP1γ and LSP2 genes at 61A and 68E, respectively, on 3L (Roberts and Evans-Roberts, 1979a; Smith *et al.*, 1981; Akam *et al.*, 1978a; Lepesant *et al.*, 1982).

What causes the initial appearance of the LSPs at the onset of the third instar is not known, but it has been suggested that ecdysone may play a role. Lepesant *et al.* (1982) demonstrated that *in vitro* cultured fat bodies showed increased synthesis of LSP2 after addition of 20-hydroxyecdysone (20-HE). It has also been demonstrated that newly eclosed adults can be stimulated to synthesise LSP2 by injection with 20-HE (Jowett and Postlethwait, 1981). The aim of this work was to determine whether the appearance of the LSP polypeptides was preprogrammed in the larval fat body or whether it occurred through a specific trigger at the onset of the third instar.

The adult hemocoel is a suitable environment for synthesis of at least one of the LSPs since synthesis of LSP2 is stimulated after ecdysone treatment. Butterworth and Bodenstein (1967) demonstrated that the larval fat body transplanted into adults could survive in the adult milieu and indeed underwent growth in the female host. This is contrary to what happens to the larval fat body surviving metamorphosis which disappears within 4 days of eclosion. Thus we decided to repeat the transplantations and determine whether LSP synthesis could be maintained or indeed initiated in the adult. The appearance of these proteins would tell us something of the natural course of events that occurs in the larval fat body during the latter half of larval life.

MATERIALS AND METHODS

LSP polypeptides persist in the young adult, although, under normal circumstances their synthesis by the larval fat body has ceased. However, it was a possibility that the transplantation of fat body into the adult would stimulate synthesis of the LSPs *de novo* by the host fat body. Therefore, to be sure that any LSPs observed after transplantation were derived from the donor fat body and not from the host, we used different electrophoretic variant strains for host and donor.

From variants previously described by Roberts and Evans-Roberts (1979a) and Akam *et al.* (1978b) strains were constructed which contained electrophoretic variants of each of the four LSP polypeptides. These strains were homozygous for either α^F or α^+; β^F or β^+; γ^0 or γ^+; 2^S or 2^+ (F and S designate fast or slow migrating polypeptides with respect to wild type, respectively, and γ^0 is a null allele). Two pairs of complementary strains were used for transplantations:

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α^F/α^F or Y; β^F/β^F; $\gamma^+ 2^+/\gamma^+ 2^+$

and

α^+/α^+ or Y; β^+/β^+; $\gamma^0 2^S$ *gl e*/$\gamma^0 2^S$ *gl e*

or

wild type (Oregon R) and α^F/α^F or Y;

β^F/β^F; $\gamma^0 2^S$ *gl e*/$\gamma^0 2^S$ *gl e*.

The $\gamma^0 2^S$ chromosome also carried the recessive mutations *glass* and *ebony*. Flies were reared on yeast/corn meal/molasses or yeast/wheat flour medium. Egg collections and rearing of larvae were at 25°C. All times mentioned refer to hours post hatch of the egg.

Fat body was dissected in a bicarbonate-buffered Ringer (3.75 g NaCl; 0.1 g $CaCl_2$; 0.1 g KCl; 0.1 g $NaHCO_3$ in 500 ml distilled water at pH 7-7.1) which was made up fresh just before use. Transplantations were performed essentially by the method of Beadle and Ephrussi (1936) using a drawn out glass capillary. Injected flies were kept as pairs. [^{35}S]Methionine (Amersham 800 Ci/mmole) was dried down before use and redissolved in Ringer; 1-2 μCi were injected per fly and the flies left for 2-4 hr. Flies were injected once more with fresh Ringer just prior to removal of the blood sample followed by immediate dissection of the carcass to recover the implant. The hemolymph sample was placed in 10 μl sample buffer for nondenaturing gels (8% sucrose/0.12 *M* Tris-citrate, pH 9.0, with bromophenol blue as running dye). Samples were kept on ice and then electrophoresed through 6.6% acrylamide (29:1, acrylamide:bis) in 0.12 *M* Tris-citrate, pH 9.0, with sodium tetraborate at 4 g/liter as running buffer. Electrophoresis was for 2 hr at 4°C and 20 mA constant current. Tissues recovered from flies were either stained with Feulgen reagent or placed in SDS sample buffer for SDS-polyacrylamide electrophoresis according to O'Farrell (1975). Gels were stained with Coomassie blue, dried, and autoradiographed.

RESULTS

The larval fat body is a single layer of cells extending the length of the body. The tissue is bilaterally symmetrical and six regions can be distinguished on each side (Rizki, 1978). The regions are not equally affected by some mutant genes so it has been suggested that all larval fat body cells may not perform the same functions.

Initially, two questions were posed. First, does larval fat body already synthesizing LSPs continue to synthesize them after transplantation into the adult? Second, do all regions of the fat body behave in the same way? Mid-third instar (72 hr post hatch) larvae of the genotype α^F/α^F or Y; β^F/β^F; $\gamma^0 2^S$ *gl e*/$\gamma^0 2^S$ *gl e* were dissected and fat body separated into four parts: region 1, the triangular piece bordering the salivary glands; regions 3 and 4, anterior to the gonad; region 5 posterior to the gonad, and region 6, the narrow dorsal strip (Rizki, 1978). The preparations were injected into 24-hr-old male and female Oregon R hosts. The following day the flies were injected with [^{35}S]methionine and after 4 hr the hemolymph was extracted and the LSPs were separated by non-denaturing polyacrylamide gel electrophoresis. In all cases labeled LSP polypeptides characteristic of the donor fat body were found in the hemolymph of the hosts irrespective of the sex of the donor or host. This confirmed that the host milieu was a suitable environment to support synthesis of the LSPs by implanted fat body. In addition each of the different regions synthesized LSPs. Thus, although we cannot be sure that every cell of the fat body synthesizes LSPs, the cells that do, must be dispersed throughout the tissue. Because of the different numbers of cells in each region, and the difficulty in determining the amount of damage caused by the dissection, in all subsequent experiments region 6 was chosen for transplantation. This region is easily defined and involved the least cell damage when removed from the rest of the fat body.

Since the host milieu maintains LSP synthesis in the implanted fat body we next transplanted fat body that had not yet started to express the LSP genes. Region 6 was taken from late second instar (46 ± 1 hr post hatch when double mouth parts are seen) and transplanted into male or female adults. The genotypes of host and donor were α^F/α^F or Y; β^F/β^F; $\gamma^+ 2^+/\gamma^+ 2^+$ and α^+/α^+ or Y; β^+/β^+; $\gamma^0 2^S$ *gl e*/$\gamma^0 2^S$ *gl e*. Reciprocal transplants were made between adults and larvae of these two genotypes. Twenty-four hours after transplantation [^{35}S]methionine was injected, 4 hr later the hemolymph collected and the polypeptides were separated on a non-denaturing gel. At the time of transplantation the donor fat body had not started to synthesize LSPs but after 24 hr in the adult abdomen LSP1 polypeptides characteristic of the donor genotype were seen in the hemolymph (Fig. 1). The LSP2 polypeptide seen in males was that of the host but in adult females both host and donor polypeptides were synthesized. This was irrespective of the sex of the donor. Transplantations which involved hosts carrying the $\gamma^0 2^S$ chromosome were only 50% as successful as those which did not. This was a consequence of the *gl e* combination. Stocks which were homozygous for this part of the chromosome are slower growing and not as vigorous as wild type. Thus, in subsequent experiments, the genotype of the donor stock was always α^F/α^F or Y; β^F/β^F; $\gamma^0 2^S$ *gl e*/$\gamma^0 2^S$ *gl e* and the host was Oregon-R.

In a similar experiment, in addition to collecting labeled hemolymph, the implant was also recovered and the pattern of protein synthesis in the tissue determined (Fig. 2). To do this the tissue had to be dissolved in SDS and then electrophoresed on a denaturing polyacryl-

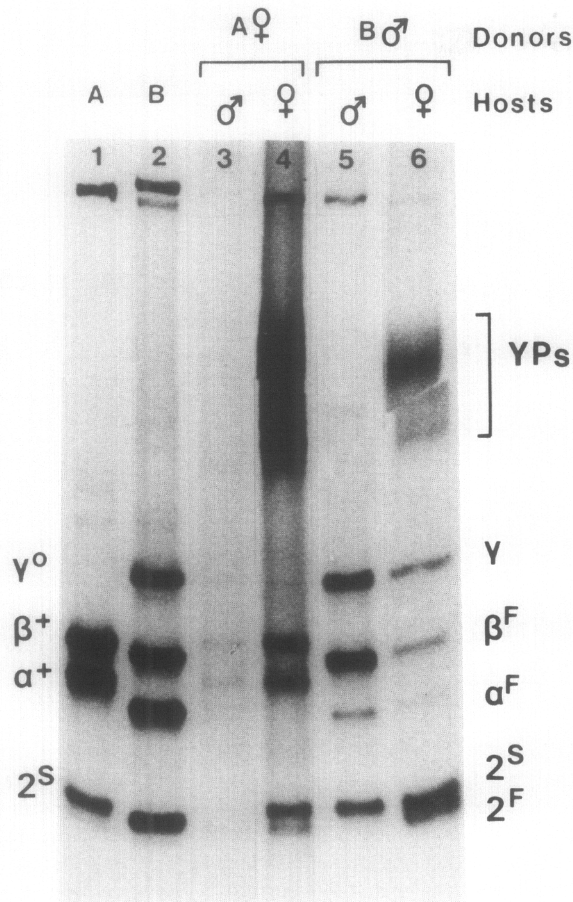


FIG. 1. Transplantation of the late second instar fat bodies into flies performed between the genotypes: (A) α^+/α^+ or Y; β^+/β^+; $\gamma^0\, 2^S\, gl\, e/\gamma^0\, 2^S\, gl\, e$ and α^F/α^F or Y; β^F/β^F; $\gamma^+\, 2^+/\gamma^+\, 2^+$. All samples were of hemolymph run on a denaturing Tris-citrate polyacrylamide gel. Tracks 1 and 2 are hemolymph samples taken from individual mid third instar larvae of the two genotypes which had been injected with 1 μCi [^{35}S]methionine 2 hr previously. Both regions 6 were taken from donor larvae of both sexes and transplanted into male and female hosts of the complementary genotypes. After 24 hr the flies were injected with 1-2 μCi [^{35}S]methionine and 4 hr later injected with Ringer and the hemolymph was collected. Each sample was run separately on the non-denaturing gel and the gel stained, dried, and autoradiographed. Four individual larvae of each genotype were used but the figure shows only one example of each. Tracks 3, 4 are samples from female donor fat bodies of genotype A in male and female adults of genotype B. Tracks 5, 6 are samples from male donor fat bodies of genotype B in male and female adults of genotype A. LSP1 polypeptides characteristic of the donor fat body are seen in every track. The sex of the donor material is reflected in the relative amounts of the α and β polypeptides because of the lack of dosage compensation of the gene in males (Roberts and Evan-Roberts, 1979b). LSP2 typical of the host is seen in every track and LSP2 from the donor is only seen in female hosts. The diffuse label found only in female host hemolymph is the yolk proteins (YPs). The quantitative differences between samples are not significant and arise through the inherent variation of the experimental manipulation.

amide gel. Under such conditions all the polypeptides of the fat body are separated but the LSPs, which are all of very similar molecular weight, migrate together, and are indistinguishable. However, by comparison of protein in the hemolymph with that in the tissue, synthesis and secretion could be examined.

The results after 24 and 48 hr of incubation in the adult abdomens were the same as the previous experiment. Synthesis of the LSP1 polypeptides typical of the donor fat body was initiated and the newly synthesized polypeptides were secreted into the hemolymph. In the tissue there were labeled polypeptides corresponding in size to those of the LSPs. LSP2, in contrast, was only synthesized by the donor fat body in the adult females and not in males, despite host synthesis of the LSP2 polypeptide being simulated by the operation. After 72 hr synthesis continued but the polypeptides were no longer secreted into the hemolymph.

In further experiments fat bodies from younger larvae were transplanted into adults. Fat bodies from 36-hr-old donors were heterogeneous with respect to LSP synthesis. The appearance of LSP1 synthesis was not always seen in both males and females. In no case was synthesis of LSP2 by donor fat body seen in adult males. For each time point at least 10 donor larvae were used, but each fat body was treated individually and no samples were pooled. For the 46-hr time point only those fat bodies in which hemolymph samples and the original implants were recovered from both male and female hosts are shown in Fig. 2. Seven out of 10 of the 36-hr-old transplants showed LSP1 synthesis despite all 10 showing an otherwise normal pattern of protein synthesis in the tissue. Thus, the absence of LSP synthesis was not because of the implant being damaged by transplantation.

In a final experiment, first instar fat body was dissected and as much of the other tissues removed as possible. The remaining fat body and some cuticle was injected into male or female adults. In five such implants recovered no LSPs were detected either secreted into the haemolymph or remaining in the tissue, despite there being an otherwise high level of protein synthesis (Fig. 3).

In recovering implants it was noticed that fat bodies incubated in female abdomens grew appreciably in size while those from males did not. This agrees with the report of Butterworth and Bodenstein (1967) who found that the average cross-sectional area of fat body cells from late second instar larvae implanted in adult males increased only after long incubation whereas in female hosts growth of fat body implants was more rapid. In the present study the question arose whether implants from younger larvae in female hosts were healthy and growing as well as implants from older donors. To compare the growth of fat body cells from first, second, and third instar donors in female hosts, recovered implants were fixed and reacted with Feulgen. The posterior region of the fat body was utilized as donor tissue for these

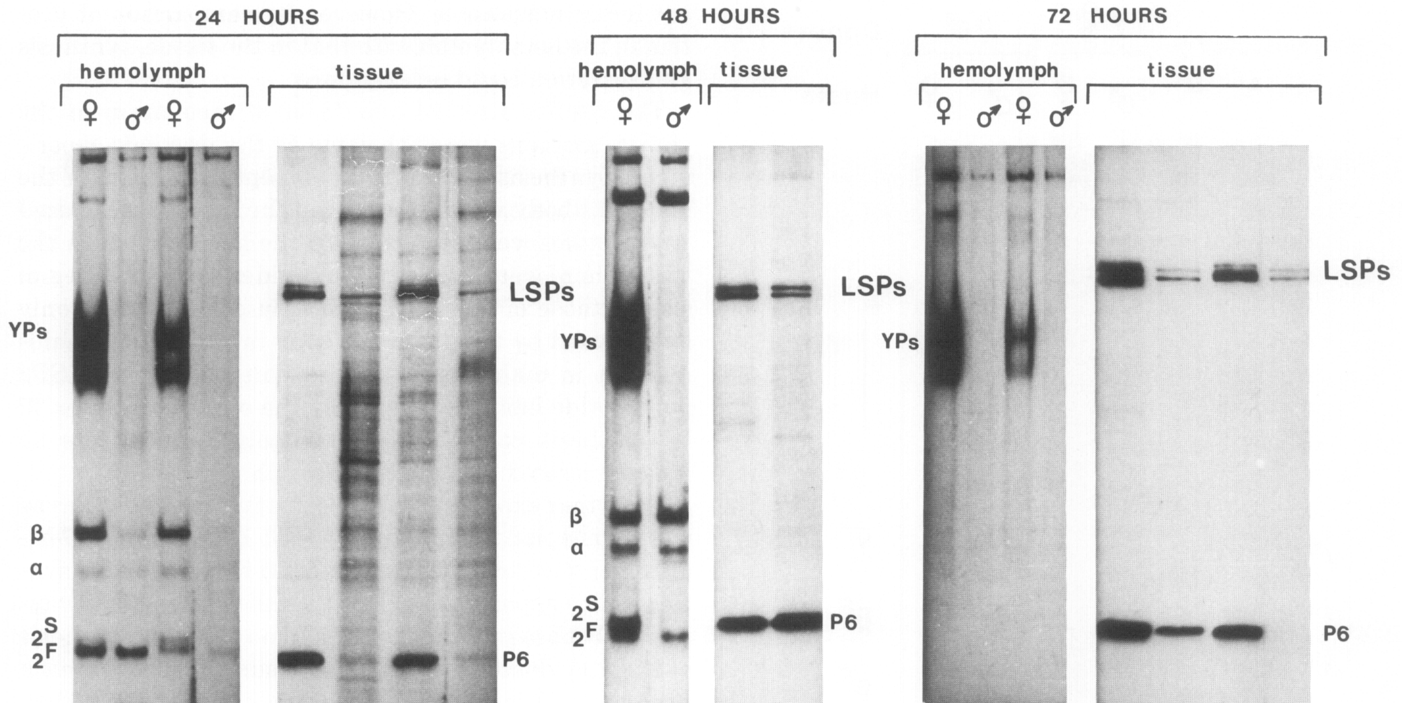


FIG. 2. Recovery of tissue and hemolymph samples following incubation in adults. Fat body from 46 ± 1-hr-old larvae was dissected and one region 6 implanted into a 24-hr-old female adult and the other region 6 from the same larva into a male. Flies were kept in pairs. After 24, 48, or 72 hr of incubation [^{35}S]methionine was injected into the abdomen and 4 hr later a sample of hemolymph collected. The fly was then dissected to recover the implant. The hemolymph was run on a non-denaturing polyacrylamide gel which separated all the LSP polypeptides. The tissue samples were dissolved in SDS and run on a denaturing polyacrylamide gel. The figure shows autoradiographs of the resulting gels. The donor fat body has the genotype α^F/α^F or Y; β^F/β^F; γ^0 2^S *gl e*/γ^0 2^S *gl e*. Hosts were Oregon R. Ten donor fat bodies were used for each period of incubation. Only representative samples are shown. LSP1 polypeptides characteristic of the donor were present in the hemolymph of both males and females after 24 and 48 hr, but were absent from the hemolymph after 72 hr. LSP2 derived from the host fat body was found after 24 and 48 hr, but not after 72 hr. LSP2 derived from the implant was found after 24 and 48 hr only in the female flies. Examination of the tissues showed that, despite the absence of labeled LSP from the hemolymph, LSPs were still being synthesized in the larval fat body. Note the YPs present in the hemolymph samples from female hosts. Also, the appearance of the protein P6 in some of the recovered implanted fat bodies.

studies so the increase in gonad size during incubation in the adult hosts could also be monitored. Photographs of implants and fat bodies from donor controls at 16, 36, 46, and 96 hr are included in Fig. 4. Several points emerged from these studies. There were no signs of degeneration or pycnotic nuclei in implants from any age group. The nuclei in implants from 16-hr donors grew and remained healthy. The nuclei of all donor groups continued to increase in size as a function of incubation time in the female hosts, however, they did not attain the size of nuclei in control fat bodies of a comparable physiological age. For example nuclei of 46 hr donor fat body after 48 hr of growth in a female host are smaller than nuclei in fat body taken from larvae at 94-96 hr. This was also true for cell size.

DISCUSSION

In performing these experiments several transplantation regimens were tried, and the ones described gave the most consistent results. There were several sources of variability in the response between individuals. In later experiments the choice of both regions 6 from the same individual donor was an attempt to minimize variation in the developmental stage of the donor tissue when comparing different hosts. Also region 6 could be the most easily dissected and injected with the minimum of damage. However, the fate of the tissue once implanted was more difficult to control. Implants could become entangled in the internal organs of the abdomen. Also injection of exactly equal amounts of label and the collection of equivalent amounts of hemolymph was not always achieved. Hence quantitative comparison of the responses of different individuals was not possible. Despite these reservations, the appearance of *de novo* synthesis of a polypeptide was highly significant.

At the end of the second instar there is no detectable synthesis of LSPs and no detectable transcripts (Lepesant *et al.*, 1982; Powell *et al.*, 1984). Our experiments show that after transfer into adults of both sexes fat

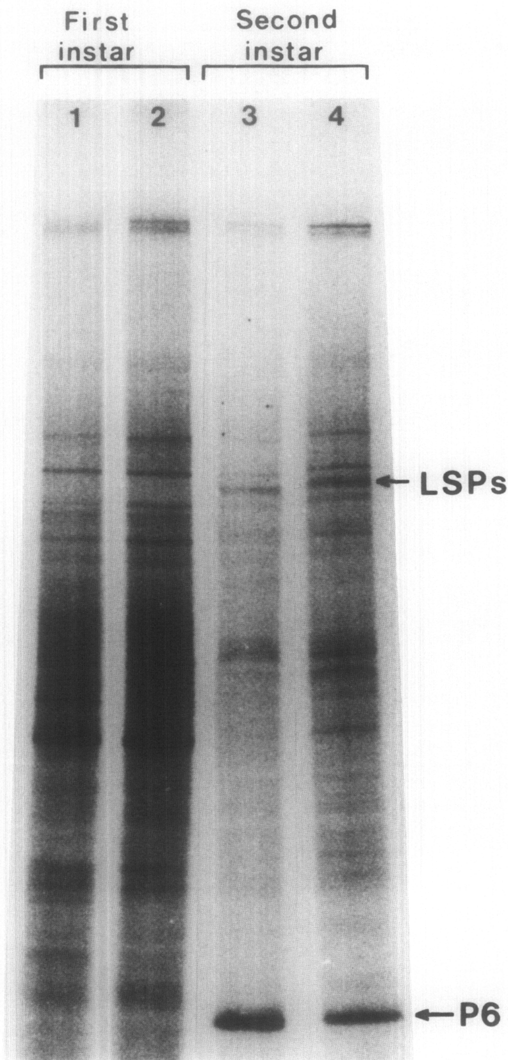


FIG. 3. Transplantation of first and second instar fat body into adult females. Fat body from 16-hr-old larvae was cultured in adult females for 3 and 4 days (tracks 1 and 2). Fat body from 46-hr-old donors was cultured in adult females for 2 days (tracks 3 to 6). [^{35}S]Methionine was injected into the flies and 4 hr later hemolymph and the implant were recovered. Both types of sample were electrophoresed as before. No LSP polypeptides derived from the implant were found in the haemolymph from flies containing first instar fat bodies (not shown). In the implants polypeptides corresponding to LSPs were only found in the second instar derived fat bodies (tracks, 3, 4, 5, and 6) and were absent from first instar implants (tracks 1 and 2). Each of the tracks contains the proteins from 1 implant. The overall pattern of protein synthesis following *in vivo* culture was much the same in both sets of implants with the exception of the polypeptide P6.

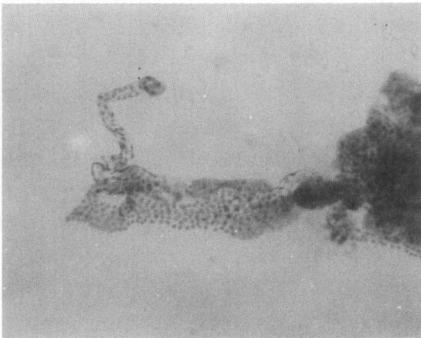
body of this age will commence to synthesize LSP1. LSP2 synthesis is initiated *de novo* in the remaining larval fat body in the host but synthesis of LSP2 in the implant is only initiated in the female host and not the male. This finding suggests that the two major larval proteins are not regulated in the same way and that the physiological milieu in the two sexes is different. LSP2 is not normally synthesized in the young adult although it is possible to stimulate *de novo* synthesis of LSP2 in the remaining larval fat body by injecting 20-hydroxyecdysone (Jowett and Postlethwait, 1981). In our experiments LSP2 synthesis was promoted in the young adult by the trauma of the transplantation itself without ecdysone. Injection of larval brain or salivary glands without fat body would stimulate *de novo* synthesis of LSP2 from host fat body. This could be as a consequence of the endogenous synthesis of ecdysone following traumatization.

The newly synthesized LSP polypeptides are secreted into the hemolymph for 48 hr. After this time, synthesis continues but the polypeptides are retained in the tissue. In this respect the implanted fat body shows some of the characteristics seen in the larva. At the end of the third instar secretion stops and uptake of the LSPs occurs (Powell *et al.*, 1984).

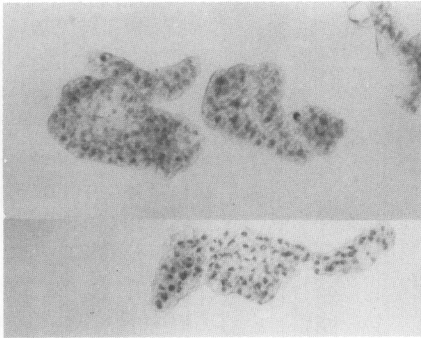
Although implants from first instar donors grew in the adult female hosts they were not shown to synthesize LSPs. The ability to synthesize these polypeptides must be acquired near the middle of the second instar (36 hr) where we found variability among donors with respect to LSP synthesis following transplant cation. This variable response suggests that at 36 hr the fat body is undergoing the changes that allow the subsequent expression of the LSP genes and these changes must be completed by 46 hr since donors of this group were more consistent in their response after transplantation. Despite the variability in LSP synthesis among the 36-hr donors, synthesis of the other fat body proteins was not impaired.

We conclude from these observations that during larval development the fat body undergoes a number of changes. It grows and increases the polyteny in the nuclei. Even first instar fat body cells will grow and undergo polytenization of the chromosomes when implanted in adult female abdomens. Examination of protein synthesis in such transplants reveals that despite the growth that occurs in female hosts the pattern of protein synthesis does not change to that of the third instar larva. However, it is apparent that a number of proteins are synthesized by the fat body throughout all the larval instars and these continue to be synthesized when first instar fat body is aged in the adult. Thus, at least part of the regulation of protein synthesis of the fat body is autonomous, but the transition from the early pattern of synthesis to that of the third instar requires some additional factor(s) which is extrinsic to the fat body and is present in the developing larva but not in the adult. During the latter half of the second instar the fat body becomes competent to synthesize LSP1 although synthesis does not commence until after the second larval ecdysis. Thus, if transplanted into the adult (male

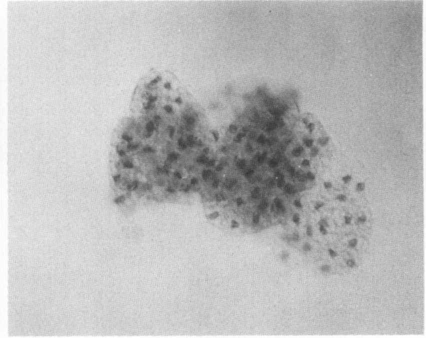
16 hr old donor
Control



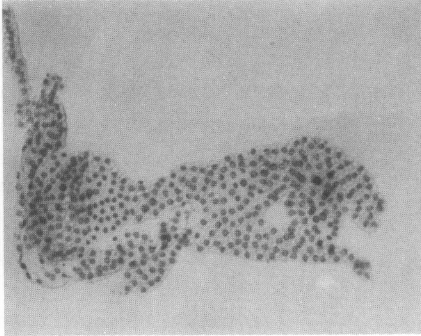
16+48=64 hr



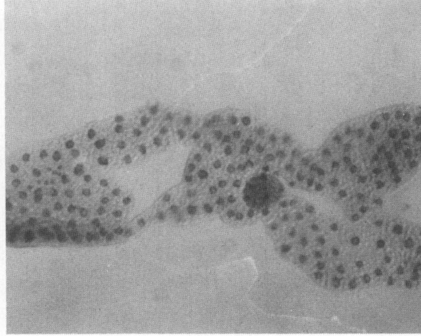
16+93=109 hr



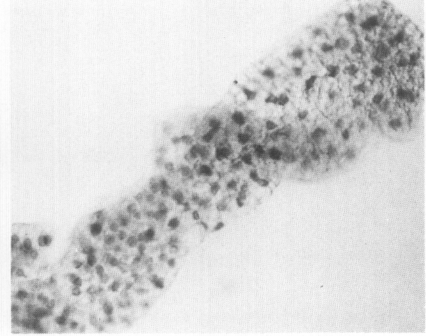
36 hr old donor
Control



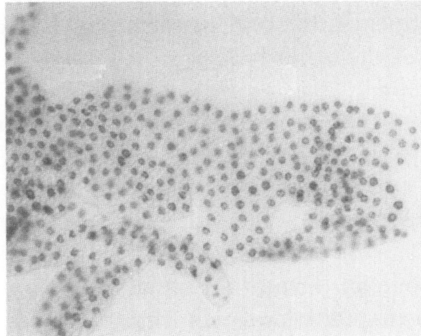
36+24=60 hr



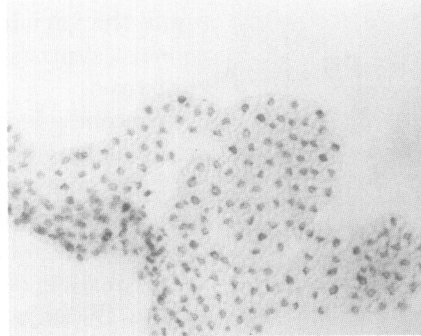
36+72=108 hr



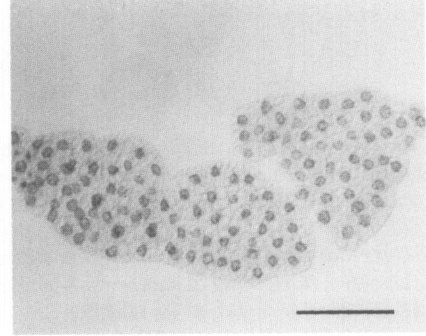
46 hr old donor
Control



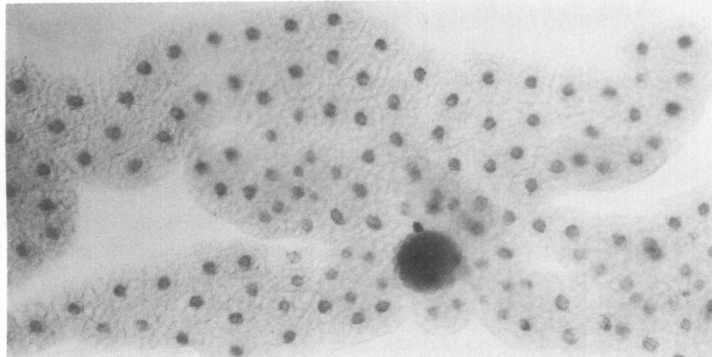
46+26=72 hr



46+48=94 hr



96 hr fat body
Control



or female) synthesis of LSP1 will occur 24 hr later. This suggests that the processes required to express the genes have been initiated. However, we cannot say whether these are acting directly on transcription and there is a delay before we see detectable levels of the proteins or whether the processes are one or more steps removed from the transcription of the LSP genes but are intrinsic to the fat body, no longer requiring the larval milieu.

The possibility that several processes may be involved is suggested by the results with LSP2. First instar fat bodies are not capable of synthesizing LSP2 if transplanted into the adult. However, fat body from 46-hr-old larvae that is able to synthesize LSP1 in both male and female adults is only able to commence LSP2 synthesis in females. Here we have the situation that only some of the changes that are a prerequisite for LSP2 expression have occurred and the adult female abdomen can provide the additional necessary factor(s) but this is absent from the male. The conclusions to be drawn are therefore that the appearance of LSP1 and LSP2 are under different controls and that at least one of the factors required for the start of LSP2 synthesis is present in the adult female and not in the male.

A third protein that is characteristic of the third instar fat body is that designated P6 by Lepesant *et al.*, (1982). They demonstrated that this low-molecular-weight methionine-rich protein appears about mid-third instar. In our experiments it was synthesized in most of those implants from 46-hr-old donors and in some from the 36-hr-old donors, but its appearance was less consistent than that of the LSPs. Even so, this suggests that the fat body was acquiring third instar characteristics.

Adult flies have long been used for the *in vivo* culture of larval tissues. Differences between the adult milieu of the two sexes is reflected in the behavior of such implants. For instance, female hosts provide a more suitable environment for culture of imaginal discs than do males. In females, discs will undergo cell division and can be maintained indefinitely by serial transplantation (Hadorn, 1963). Imaginal discs cultured in males do not grow and gradually lose their ability to respond to metamorphosing factors. Since the growth of discs depends on the presence of molting hormone supplied by the ring gland and since the ring gland degenerates during pupal life it seems reasonable to assume that the growth restricting environment of the adult male is one in which the molting hormone is absent. This is supported by the fact that ring gland transplants into adult disc-carrying males induce growth in the transplanted discs (Schweizer and Bodenstein, 1975).

Larval salivary glands may also be cultured in the abdomens of adult flies for considerable periods of time (Bodenstein, 1943). Such transplanted glands replicate their DNA, often forming very large polytene chromosomes (Hadorn *et al.*, 1963). Considerable changes in puffing of the transplanted glands occur depending on the age of the donor gland and the age and sex of the host (Staub, 1969). The puffing pattern although showing some similarities with that of the larva is generally abnormal. However, on exposure to exogenous ecdysone glands whose chromosomes have reasonably normal morphology will show a characteristic early hormonal response (Ashburner and Garcia-Bellido, 1973).

As described here, the differential response of fat bodies cultured in male and female hosts also reflects differences in the milieu of the two sexes. Fat bodies grew considerably more in females than in males. This was previously reported by Butterworth and Bodenstein (1967) who also showed that in males the larval ring gland or the ovary would stimulate growth, protein deposition, and histolysis in larval fat bodies when coinjected with the implants. With this in mind it is interesting to speculate whether the *de novo* synthesis of LSP2 will occur in males which have received either the ovary or the ring gland. Several attempts to simulate LSP2 synthesis by donor fat body in male hosts by 20-HE have been unsuccessful in our hands.

Failure to obtain LSP synthesis with first instar fat body suggests that the priming must occur between 16 and 46 hr post hatch. Thus, this period is one of particular interest with respect to expression of the LSP genes. The nature of the changes which the fat body undergoes in this period is unknown but one can speculate they will involve alterations in the chromatin configuration which will make the genes accessible for transcription. Preliminary work to examine changes in chromatin configuration during development has begun (Jowett, 1985). Each of the active LSP genes in the fat body has a different configuration from the inactive genes in embryo nuclei. Clearly, the period during larval fat body differentiation when these configurational changes are

FIG. 4. Feulgen stained fat body. To compare the changes of fat body nuclei during incubation in adult female abdomens, fat bodies from 16-, 36-, 46-, and 96-hr-old larvae were fixed and other groups of fat body from 16-, 36-, and 46-hr-old larvae were transplanted in adult females. Implants were recovered and fixed at the times indicated. The tissues were reacted with Feulgen and mounted on microscope slides. Fat body from donors of all ages grew in female hosts. However, nuclei and cells in the implants were smaller than those in controls at a comparable age. After 93 hr in the female host, 16-hr fat body cells were as large as those of 46-hr donors that had remained in hosts for 26 hr. The latter synthesized LSPs whereas the former did not. The bar represents 100 μm for all photographs.

brought about is of significance in relation to the findings presented here. Experiments to correlate the physiological changes with the molecular rearrangements of the chromatin are currently under way.

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