MUTANT GENES REGULATING THE INDUCIBILITY OF KYNURENINE SYNTHESIS

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ABSTRACT

Alterations in the cellular synthesis of kynurenine in the larval fatbody of *Drosophila melanogaster* may be obtained by feeding the precursor tryptophan or by changing the genotype. In the wild type *Ore-R* strain, autofluorescent kynurenine globules normally occur in the cells in the anterior regions of the fatbody designated as regions 1, 2, and 3. When tryptophan is included in the larval diet, kynurenine will develop throughout the entire fatbody, thus extending to the cells in regions 4, 5, and 6. In the fatbodies of both the *sepia* mutant strain and the mutant combinations of the suppressible *vermilion* alleles with the *suppressor* gene (su^2-s, v^1 and su^2-s, v^2), kynurenine is found in the cells from region 1 through region 4. This involvement of additional cells in the synthesis of kynurenine occurs under the usual culture conditions for *Drosophila*. When *sepia* larvae are fed tryptophan, kynurenine appears in all of the cells of the fatbody. However, dietary tryptophan does not induce kynurenine production in cells in regions 5 and 6 in the mutant combination su^2-s, v^1 or su^2-s, v^2. In the latter strains, an increase in the quantity of kynurenine in the fatbody is detected, but this increase remains limited to the same cells in which kynurenine production is found under normal feeding conditions. When the v^{36f} allele is combined with the su^2-s allele, an extremely faint autofluorescence characteristic of kynurenine is found in some of the anteriormost fat cells of regions 1 and 2. This autofluorescence becomes intensified when tryptophan is fed to su^2-s, v^{36f} larvae. The genetic control of kynurenine synthesis in the cells of the fatbody of *Drosophila melanogaster* has been previously demonstrated. The present observations establish genetic regulation of the ability to induce kynurenine production within a cell through the administration of the inducer tryptophan. Kynurenine production has been considered as a unit function of the cell as a whole rather than of the enzyme alone, and it has been concluded that even though cells in different parts of the body perform this same function (kynurenine production), the gene loci regulating this function may be different for cells in different regions of the body. A phenomenon of overlapping domains of gene actions at the cellular level offers a genetic and cellular basis for developmental and physiological homeostasis.

The natural fluorescence of kynurenine provides a convenient method to localize this metabolite intracellularly through the use of fluorescence microscopy. Examination of the fatbody of wild type *Ore-R* prepupae of *D. melanogaster* with the fluorescence microscope has revealed that kynurenine is restricted to the cells in the anterior region of this tissue (5). However, induction of kynurenine synthesis in the remaining cells of the larval fatbody can be achieved by feeding the precursor tryptophan to *Ore-R* larvae (9). These features, together with the availability of mutant genes which affect the synthesis of kynurenine in the larval cells of *Drosophila*, offer an excellent system

to study the genetic regulation of cellular differentiation.

The *vermilion* gene blocks kynurenine synthesis, and no autofluorescent kynurenine globules appear in the fat cells of *vermilion* mutant larvae. However, combination of the mutant factor *suppressor*^{2} with the *vermilion* gene restores kynurenine synthesis in the fat cells (6). This restoration involves the participation of the anterior fat cells, the normal site of kynurenine accumulation in the wild type *Ore-R* strain under the usual conditions of culture, as well as cells located posterior to this region. A number of *vermilion* alleles have been described in *Drosophila*, and these alleles have been classified as suppressible or unsuppressible on the basis of their response to the non-allelic *suppressor* mutation (1, 2, 10). This classification of the genetic suppression of *vermilion* by the *suppressor* gene has been determined by the appearance of the brown component of eye pigment which is absent in *vermilion* mutants. As shown previously, kynurenine, a precursor of the brown component of *Drosophila* eye pigment, appears in the fatbody of the suppressible *vermilion* mutant when this is combined with the suppressor gene (su^2-*s*, *v*). Further examination of the combinations of various *vermilion* alleles with the mutant gene *suppressor*^{2} is the subject of the present report, as well as a study of the induction of kynurenine synthesis in the fat cells by feeding tryptophan to these mutant strains.

PREPARATION OF GENETIC STOCKS

For the previous examination of *vermilion* alleles and the suppressor genes, mutant strains had been obtained from three different laboratories, and establishment of the autofluorescent pattern for a particular allele or combination of genes involved comparison of stocks obtained from different sources. For the present study, standard stocks were prepared which would be comparable with respect to their genetic background. This factor is important in the type of autofluorescent studies undertaken, as well as the necessity of removing all body and eye color mutants from the original stocks containing the mutant gene under study. The autofluorescence of the fatbody is affected by mutant genes influencing body color as well as mutant genes altering both the red and brown components of the eye pigments, and presumably precursors utilized in all of these systems are occurring in the fatbody.

Three *vermilion* (*v*) pseudoalleles in combination with *suppressor*^{2} (su^2-*s*) have been examined in the present study, v^1 and v^2 which are homoallelic, and v^{36f} which is heteroallelic and separable by crossing-over from v^1 and v^2. The latter alleles are suppressible whereas the v^{36f} allele has been designated as unsuppressible. The su^2-*s* mutant allele from a stock of su^2-*s*, v^{36f} was separated and combined with the v^1 and v^2 mutant alleles, and the reconstituted su^2-*s*, v^1 and su^2-*s*, v^2 males were crossed to attached-X females carrying *yellow* body and *forked* bristles as markers (*yf*: =). The background of this particular attached-X stock had been changed previously be repeated backcrossing of the attached-X females to *Ore-R* males. When su^2-*s*, v^1 or su^2-*s*, v^2 males are crossed to attached-X females, a stock is perpetuated in which the X chromosome carrying the su^2-*s* gene and the *v* gene travels from father to son. The stocks in which the X chromosomes are being compared are thus standardized against the *Ore-R* autosome background and crossing-over in the X chromosome is inhibited. The v^{36f} allele was also placed in a stock perpetuated as su^2-*s*, v^{36f} ♂♂ X *yf*: = ♀♀. The presence of the designated mutant alleles in each stock was appropriately verified by genetic crosses. Throughout the present study, observations have been reported for male larvae since only the males contain the X chromosome carrying the su^2-*s* and *v* mutant genes.

PREPARATION OF MATERIAL

The methods for collecting and raising *Drosophila* larvae were the same as those reported previously (4). The experiments consisted of examination of the fatbodies of larvae and pupae which had been maintained under three feeding conditions. Some larvae were maintained on cream of wheat medium seeded with yeast throughout their development. Other groups of larvae were transferred to washed Whatman cellulose powder at 66 hours of age. The cellulose powder was then moistened either with a 0.03 M solution of L-tryptophan or with distilled water used as the control solution. The fatbodies of late third instar and bubble stage pupae were dissected in Ringer's solution and examined with a Zeiss fluorescence microscope equipped with apochromatic optics and a darkfield ultracondenser. Separation of kynurenine from isolated fatbodies was achieved by thin layer chromatography with Silica Gel G. Plates were developed for 35 minutes in butanol:acetic acid:water (13:3:4) and examined with ultraviolet light prior to spraying with Ehrlich reagent.

A microspectrofluorometer was assembled to obtain

fluorescence spectra of freshly dissected fat cells and fat cells following incubation. The exciting light at wavelength 365 mμ was passed through the specimen on the microscope slide. The emitted light was focused at the entrance slit of a monochromator provided with a cam drive and a mounted IP21 phototube at its exit slit. The signals generated from the phototube were transmitted through a photomultiplier microphotometer and fed into the oscilloscope. Calibration of the wavelengths was checked at each run by using a standard didymium filter and a tungsten light source. A detailed description of this instrument will be reported elsewhere.

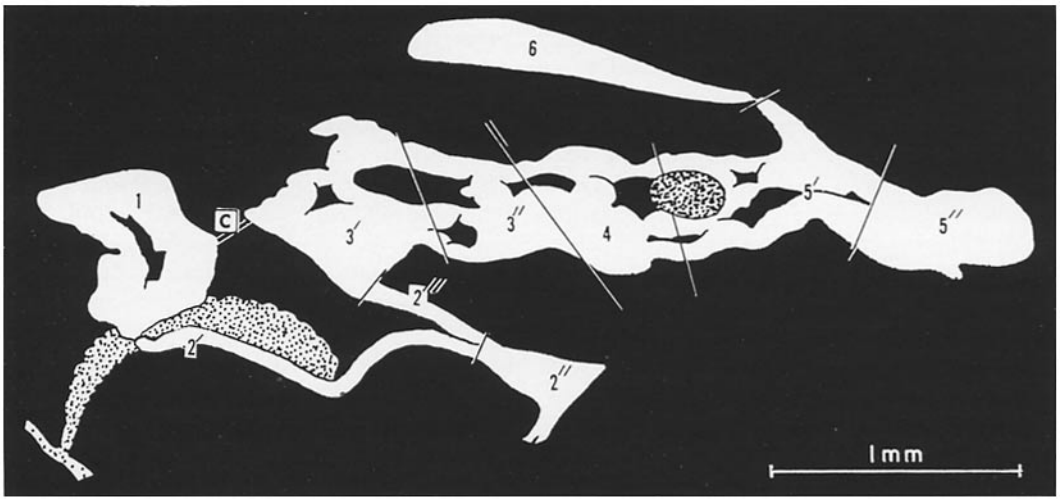


FIGURE 1 A camera lucida drawing of the left lateral half of the fatbody of a late third instar larva. The anterior region *1* is the mass which is located dorsal to the salivary gland, *2′* and *2″* are the strands and the ventral triangular piece forming a commissure connected to the fat mass of the right side of the body, and *2‴* is the recurrent strand rising forward and joining the anteriolateral fat mass *3′*. In the *Drosophila* larva the fatbody shows a dimorphism in both the male and female; this is represented by the presence or absence of a small strand of approximately 20 cells connecting region *1* and *3′* at point *C*. (The anterior stippled structure is the salivary gland, and the stippled structure between regions *4* and *5* is the testis.)

OBSERVATIONS

The cell population of the larval fatbody of *D. melanogaster* is composed of one morphological type. On the basis of their fluorescent content, the cells from the different regions of this fat mass can be differentiated. In previous reports (7, 9) the regions of the fatbody were referred to an anterior and posterior, or anterior, mid, and postgonadal. This subdivision, which was based on the observations of the fluourescence of the cells in the various regions of the fatbody, proved inadequate to express the degree of regionalization of fluorescence apparent in the cells of the fatbodies of the genetic strains examined for the present report. The method adopted for numbering the regions of the fatbody is presented in Fig. 1 with a camera lucida drawing of a lateral half of the fatbody.

The light blue fluorescence characteristic of kynurenine extends from regions 1 through region 3 in the *Ore-R* wild type fatbody when larvae are raised on the regular medium with yeast or transferred to cellulose powder soaked with distilled water from 65 hours of age until pupation. As described previously, kynurenine will appear in the cells of the entire fatbody (regions 1 to 6) of *Ore-R* larvae if they are transferred to cellulose powder soaked with 0.03 M L-tryptophan at 65 hours of age. Continuing the use of the present designation, region 4 indicates the area to which fluorescence extends in mutant larvae su^2-*s*, *v* (6).

Light blue fluorescent globules characteristic of kynurenine localization do not appear in the fat cells of any of the *vermilion* alleles, v^1, v^2, v^{36f}, nor are they visible when larvae of these strains are transferred to distilled water, or tryptophan solution at 65 hours of age. In the normal strain, sky blue fluorescence identified as kynurenine is

localized in globular inclusions, and the fluorescence of these cellular structures is readily viewed when the cellular contents are released upon rupture of the cell membrane. The intensity of this fluorescence is not altered during examination with ultraviolet light. When fat cells of *vermilion* larvae are ruptured, deep blue and yellow fluorescent substances are released. Some of these materials fade and undergo color changes upon exposure to ultraviolet light, but the fluorescence of the globules does not resemble that found in the anterior cells of the normal *Ore-R* strain. When the su^2-*s* allele is combined with the v^1 or v^2 alleles, suppression of the mutant effect of the *v* alleles is evidenced by the appearance of the autofluorescence characteristic of kynurenine in the fatbodies of the su^2-*s*, v^1 and su^2-*s*, v^2 larvae. Light blue fluorescent material extends from the anteriormost cells to the level of the gonads, and that this material is kynurenine has been verified by chromatographic analysis. The observations on these mutant combinations thus confirm the previous study of a mutant stock containing su^2-*s* and a suppressible *vermilion* allele, *v* (6).

Larvae from each of the mutant combinations of *suppressor* with the suppressible *vermilion* alleles were transferred to 0.03 M tryptophan solution beween 65 to 70 hours of age. The larval fatbodies were dissected from bubble stage pupae and examined with the fluorescence microscope. Kynurenine fluorescence in the larval fatbodies of su^2-*s*, v^1 and su^2-*s*, v^2 pupae extended from region 1 to region 4, but none could be detected in the postgonadal cells. Three successive feeding experiments confirmed these observations. An increased quantity of tryptophan (0.04 M, 0.05 M, 0.06 M) did not result in any light blue fluorescence in the postgonadal cells of regions 5 and 6; the fluorescence characteristic of kynurenine remained limited to the cells in regions 1 through 4. The distribution of kynurenine, therefore, remained the same as when these larvae were raised on a normal yeast diet, but the intensity of the fluorescence in these cells appeared to be considerably increased on tryptophan solution. In the control specimens of su^2-*s*, v^1 and su^2-*s*, v^2 placed on water at 65 hours of age, kynurenine was not detectable in region 4 but was found only in regions 1, 2, and 3. A quantitative comparison of the fluorescence attributable to kynurenine under different feeding conditions was obtained by chromatographic separation. For each experiment fatbodies were dissected from 4 pupae (su^2-*s*, v^1 and su^2-*s*, v^2) raised on tryptophan, 4 pupae raised on normal food, and 4 pupae maintained on water in the control dishes. In each instance, a larger quantity of kynurenine was obtained from tryptophan-fed larvae. For some experiments, the fatbodies (su^2-*s*, v^2) were dissected to include only regions 1 through 4 to demonstrate that the increased kynurenine obtained after tryptophan feeding was the product of these cells.

The v^{36f} allele has been classified as an unsuppressible allele. However, a faint autofluorescence characteristic of that associated with kynurenine can be detected in the anteriormost cells of the fatbodies of su^2-*s*, v^{36f} pupae. This fluorescence is barely detectable, and it is, therefore, difficult to establish its exact cellular distribution which appears to be limited to fewer cells than the normal pattern in the *Ore-R* strain. When su^2-*s*, v^{36f} larvae were given 0.03 M L-tryptophan, the light blue fluorescence characteristic of kynurenine was apparent in the fatbody regions 1 and 2′ (Fig. 1). Higher concentrations of tryptophan did not result in the appearance of kynurenine fluorescence beyond the cells in regions 1 and 2′.

During a survey of the fluorescent pattern of the fatbodies of other mutant strains of *Drosophila*, it was noted that the eye color mutant *sepia* (*se*) differed from the normal *Ore-R* strain with respect to the regions of the fatbody which show kynurenine fluorescence. The fluorescence characteristic of kynurenine extends from region 1 through region 4, thus including additional cells in a manner similar to that resulting in su^2-*s*, v^1 and su^2-*s*, v^2. The *sepia* mutant does not behave as a suppressor of *v*, for kynurenine production is blocked in *se*, *v* larvae. When *sepia* larvae are given 0.03 M tryptophan, the fluorescence characteristic of kynurenine extends throughout the fatbody as in *Ore-R* wild type larvae. The alteration in the distribution pattern of kynurenine in *sepia* larvae is not correlated with an effect on the inducibility of kynurenine as noted in the suppressor strains.

The attached-X females in the stocks used in the present study showed the same pattern of kynurenine autofluorescence in the fatbody as wild type specimens when raised on normal food. In the feeding experiments with tryptophan, kynurenine was produced in all of the cells of the fatbody of the attached-X females, in contrast to the limited distribution of kynurenine found in the su^2-*s*, *v* males which were feeding in the same

dishes. This induction in the fat cells is the expected response for the attached-X genotype since the wild type alleles at the *v* and *su*^{2}-*s* loci are present. In some experiments, male larvae were separated from female larvae within 2 hours after hatching. These larvae were then raised under the various experimental conditions; no differences were noted in the results of these experiments as compared to those in which the attached-X females were raised in the same dishes.

Tryptophan pyrrolase activity has previously been demonstrated in the fatbody of *Drosophila*. In larvae shortly before pupation, the detectable tryptophan pyrrolase activity was restricted to cell-free extracts of isolated fatbodies, and this activity was increased by feeding tryptophan to the larvae (8). An increase in the number of fat cells displaying the autofluorescence characteristic of kynurenine after tryptophan feeding is correlated with increased enzyme activity under these conditions. Furthermore, isolated groups of cells from fatbody region 4 of normal larvae developed the characteristic fluorescence of kynurenine when these cells were incubated under *in vitro* conditions in the presence of tryptophan, thus indicating that these cells are potentially capable of converting tryptophan to kynurenine although they do not perform this function under the usual conditions of culture. These previous *in vitro* studies were limited to examination of incubated tissues by fluorescence microscopy to determine whether the fluorescence of the cells resembled that of kynurenine or not. Verification of this identification has been confirmed by adoption of two additional techniques, thin layer chromatography and microspectrofluorometry, which are suitable for the small quantities of material available. For all of these studies on *Ore-R* normal larvae, the area of fatbody designated region 5 has been used. Incubation of the tissues in an O_2 atmosphere proceeded from 45 to 90 minutes for different groups of experiments. Tissues from 4 larvae were incubated in Ringer's solution containing tryptophan while incubation of this number of control fatbodies was performed in Ringer's solution. The chromatographic plates were examined with ultraviolet light and subsequently sprayed with Ehrlich reagent. The light blue fluorescent spots on the chromatograms prepared from tissues incubated with tryptophan corresponded in Rf to the known samples of kynurenine. Similar to the kynurenine samples, these spots turned orange after spraying with Ehrlich reagent. Kynurenine was not detected in any of the posterior fat tissues following incubation in Ringer's solution nor in freshly dissected posterior fat cells.

A microspectrofluorometer has been utilized to obtain fluorescence spectra of individual cells in order to further characterize the fluorescence emitted by the cells from various regions of the fatbody. The light source in this instrument is the HBO 200 burner and filter system isolating the 365 mμ band which has been used for all of the microscopic examinations of fluorescence on the fat cells. This wavelength corresponds to the principal excitation wavelength for kynurenine in solution at pH 7 as determined in an Aminco-Bowman spectrophotofluorometer. In order to establish the emission spectrum of a known sample of kynurenine within fat cells, *vermilion* fatbody was placed in Ringer's solution containing kynurenine. These treated cells fluoresce with the characteristic sky blue autofluorescence observed in *Ore-R* fatbody when they are examined with the fluorescence microscope, and the emission spectrum of kynurenine within the *vermilion* fat cells with a peak at 465 to 470 mμ is identical to that of the anterior fat cells of *Ore-R* larvae. This peak is absent in the posterior fat cells (refer to Fig. 2, curves *1*, *2*). When *Ore-R* larvae are fed tryptophan, cells from all regions of the fatbody show this same emission spectrum (curves *3*, *4*). Following *in vitro* incubation the posterior fat cells of normal larvae contain this fluorescent material (curves *5*, *6*). A similar emission spectrum is obtained from the region 1 cells of *su*^{2}-*s*, *v*^{36f} larvae, but this fluorescence does not extend even as far as region 3 following tryptophan feeding (curves *7*, *8*). On the other hand, curves *9* and *10* demonstrate that this material extends through regions 1 to 4 in *su*^{2}-*s*, *v*^{2} larvae.

DISCUSSION

Modifications in the number of cells which contain kynurenine in the larval fatbody of *D. melanogaster* can be achieved by alterations in the genetic make-up or through changes in the quantity of precursor included in the larval diet. In the wild type *Ore-R* strain only the cells in the anterior region of the fatbody show an accumulation of fluorescent kynurenine globules (5). Kynurenine will appear in the cells throughout the fatbody of this strain, however, if the larvae are given tryptophan. The extent of the induced

kynurenine fluorescence is determined by the quantity of tryptophan fed to the larvae as well as the age at which the treatment is begun and the duration of the feeding period of tryptophan. Through the manipulation of these factors, it is possible to achieve a varying number of cells displaying the autofluorescence of kynurenine, with a range extending from the limited normal pattern to the total cell population of the fat tissue. In studying the variations of kynurenine distribution in the fat cells of mutant strains, the optimum conditions for obtaining maximum cellular response in the normal strain have, therefore, been utilized. These optimum conditions do not effect total cell activity in the suppressed *vermilion* strains, nor do concentrations of tryptophan in excess of that required for the *Ore-R* normal strain induce an increase in the active cell population in these mutant strains. The fluorescent pattern of kynurenine distribution in the fatbodies of su^2-s, v^1 and su^2-s, v^2 pupae remains limited to those cells in which it appears in these strains on the normal yeast diet. There appears to be an increase in the fluorescence of the cells involved with a higher concentration of tryptophan feeding, but this increased activity does not extend to additional fat cells. The same phenomenon appears to occur with the combination of the v^{36f} allele and the su^2-s mutant gene. The faint autofluorescence of kynurenine appearing in the anteriormost fat cells of su^2-s, v^{36f} larvae becomes more pronounced when tryptophan is fed to these larvae. The present observations on cell fluorescence offer further evidence that the difference between the unsuppressible v^{36f} allele and the suppressible *vermilion* alleles is one of a quantitative nature rather than absolute. Shapard (10) reported a decrease in tryptophan accumulation when the v^{36f} allele was combined with suppressor, and Kaufman (3) detected slightly higher tryptophan pyrrolase activity in su^2-s, v^{36f} adults than in v^{36f} adults.

An adaptive increase in tryptophan pyrrolase activity is obtained in the fatbody of the *Ore-R* strain of *Drosophila* through the administration of tryptophan in the diet (8). The autofluorescence characteristic of kynurenine is limited to cells in the anterior region of the fatbody when larvae are raised under normal culture conditions, but this fluorescence appears in posterior fat cells when the larvae have been fed tryptophan (9). The modification in the number of cells containing kynuren-

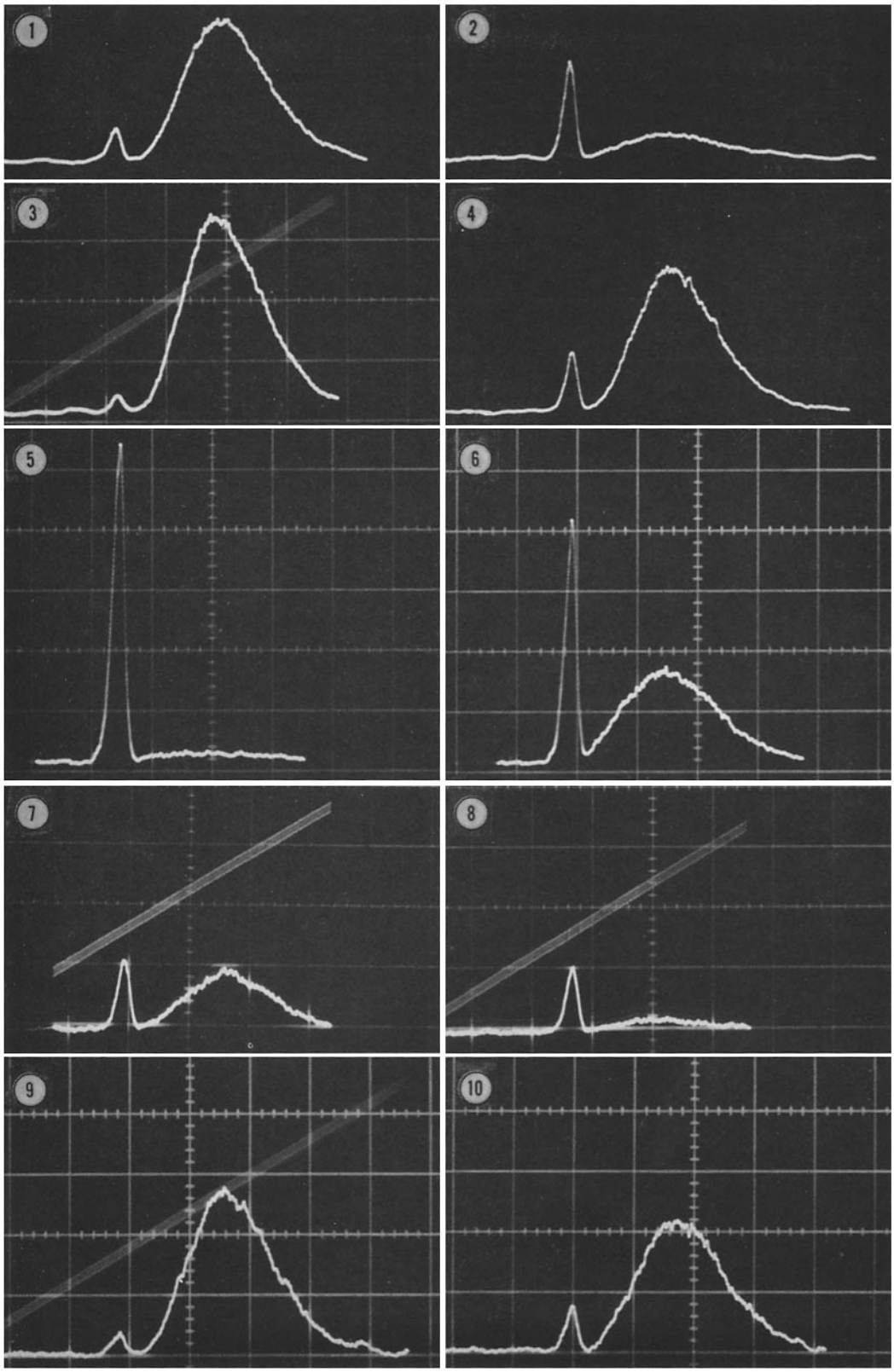
FIGURE 2 Fluorescence emission spectra of 10 μ diameter plugs through individual fat cells which measure approximately 50 μ in diameter. The upper beam on the oscilloscope is receiving signals from the cam drive of the monochromator and appears as the rising straight line in the photographs, while the lower beam describes the signals from the photomultiplier microphotometer. The time base is set with the beams moving from left to right at a rate of 5 sec/cm. The peak to the left in each curve represents the excitation peak at 365 mμ, and to the right lies the area of the fluorescence emission energy spectrum of the fat cells. Curves *5* to *10* were recorded at an increased sensitivity (0.001) as compared to a setting of 0.003 for curves *1* to *4*.

Curves *1* and *2* are fluorescence spectra recorded for an anterior fat cell (region 1) and a posterior fat cell (region 5) respectively from the same male *Ore-R* specimen. Curve *2* represents the background fluorescence for the fat cells; decay of fluorescence can be noted for some of these materials in the cells during exposure to UV. Kynurenine fluorescence is stable during this exposure. As described in the text, the fluorescence spectrum of *vermilion* fat cells which have been placed in a sample of kynurenine is the same as that of curve *1*. Curves *3* and *4* are spectra for the anterior (region 1) and posterior (region 5) fat cells, respectively, from an *Ore-R* male prepupa which has been fed tryptophan.

Curve *5* is the spectrum through a region 5 fat cell of an *Ore-R* female following incubation in Ringer's solution. The background fluorescence in posterior fat cells is less than in male specimens and, therefore, females have been used for some of the incubation studies in which the quantity of kynurenine produced is small. Curve *6* is the fluorescence spectrum of a fat cell from the corresponding region of the other lateral half of the same specimen following incubation in Ringer's solution with tryptophan.

Curve *7* is the spectrum of region 1 fatbody from a su^2-s,v^{36f} specimen which has been fed tryptophan, and curve *8* is recorded through region 3 of the same specimen.

Curve *9* is the spectrum of region 1 fatbody from a su^2-s,v^2 specimen which has been fed tryptophan, and curve *10* is the spectrum of region 4 fatbody from the same specimen.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ine is thus correlated with increased activity of an inducible enzyme. These observations are of value to an understanding of cell activity and cell differentiation if the observed localization of the fluorescent cellular product, kynurenine, represents synthetic activity of the cell in which this product is localized rather than accumulation of a metabolite synthesized elsewhere in the body. Previous *in vitro* studies with a group of posterior fat cells indicated that the fluorescence characteristic of kynurenine appears in these cells following incubation with tryptophan (6). The observations have been extended to include the remaining regions of the posterior fatbody, and chromatographic separation of kynurenine from posterior fat cells following *in vitro* incubation confirms the identification of the cellular fluorescence as due to kynurenine. Fluorescence spectra offer additional evidence that the fluorescent material appearing in the posterior fat cells following incubation with tryptophan corresponds to that which normally appears in the anterior fat cells and which can be induced *in vivo* in the posterior fat cells by feeding tryptophan.

That the potential of individual fat cells to respond to induction by the precursor tryptophan is genetically controlled can be demonstrated in the fat cells by utilizing mutant strains in which the regulation of this cell activity is affected. When tryptophan is fed to su^2-s, v^{36f} larvae, a small group of fat cells (regions 1 and 2′) contain detectable kynurenine fluorescence whereas on normal food kynurenine is barely detectable in these fat cells in this mutant strain; the same quantity of tryptophan supplied to su^2-s, v^1 or su^2-s, v^2 larvae does not induce kynurenine production in any cells in which it does not appear under normal feeding conditions, and kynurenine thus remains restricted to the cells of regions 1 through 4 of these genotypes; kynurenine will appear in all of the fat cells (regions 1 to 6) of *Ore-R* wild type and *sepia* mutant larvae given this quantity of tryptophan. Under normal feeding conditions, kynurenine occurs in fewer larval fat cells of the wild type *Ore-R* strain than in the *sepia* mutant strain in which cells of the 4th region are active. Only when the expression of the potential for kynurenine production is viewed at the level of the individual cell can the responsive system be adequately evaluated. Over-all increased kynurenine production may be achieved by greater activity within a specific group of active cells, or it may result from the stimulation of additional cells contributing to this function.

The differentiation of an individual fat cell as a "kynurenine cell" is specified by genetic factors which define the norm of reaction for the cell in relation to an inducing substance. A sufficient increase in intake of tryptophan can stimulate all of the fat cells in the *Ore-R* wild type strain to produce kynurenine. Substitution of mutant alleles at the *vermilion* and *suppressor* loci alters the norm of reaction and a differential is established between groups of cells. This regional differentiation involves a restriction in the inducibility of kynurenine synthesis in some of the fat cells, and the regions affected depend upon the status of the *vermilion* pseudoallelic locus included in the genotype. In the presence of excess dietary tryptophan, both the suppressible *vermilion* and the unsuppressible *vermilion* alleles react to the same modifier (su^2-s) but the interactions of these genes specify activity in different cells. The domain of influence of the combinations su^2-s, v^1 and su^2-s, v^2 differentiate the cells at the level of the gonad whereas the su^2-s, v^{36f} domain demarcates a small group of cells in the anteriormost region of the fatbody from the remaining fat cells. From the present observations, an interesting property of the cell as an individual unit within a multicellular framework emerges, for it becomes apparent that even though cells in different parts of the body may perform the same function (in this instance, kynurenine production), the gene loci regulating this function may be different for different cells. This explanation incorporates the observations made on the fat cells of various mutant strains, and indicates the desirability of uncovering the mechanisms whereby the differential states among the cells are achieved and maintained. Manifold interactions must be included in the final analysis, both as concerns the individual fat cell within the fatbody and the physiological interrelationships of this tissue with other organ systems in the body. A phenomenon of overlapping domains of gene actions at the cellular level as viewed in the larval fatbody may provide a genetic and cellular basis for developmental as well as physiological homeostasis in multicellular organisms.

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