CYTODIFFERENTIATION IN THE *ROSY* MUTANT OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

In the *rosy* mutant of *Drosophila melanogaster*, two types of autofluorescent cytoplasmic inclusions are found in the cells of the posterior region of the fatbody at the prepupal stage. Bright yellow autofluorescent granules accumulating within larger inclusions clearly demarcate this area of the fatbody which also contains cobalt blue fluorescent globular material. Such inclusions were not noted in the normal *Ore-R* strain at this stage nor in the series of mutant strains examined other than the *rosy*^{2} and *maroon-like* mutants. The pattern of biochemical deviation of the latter two mutants is known to be identical to that of the *rosy* mutant, and a portion of this mutant upset can be ascribed to the absence of xanthine dehydrogenase. These mutants lack the products of enzyme activity, uric acid and isoxanthopterin, and accumulate their precursors, hypoxanthine and 2-amino-4-hydroxypteridine. Chromatographic studies on the fatbody of *rosy* prepupae have shown that 2-amino-4-hydroxypteridine is limited to the posterior region; this correspondence in location as well as color of fluorescence indicates that the cobalt blue autofluorescent globules in the fatbody contain 2-amino-4-hydroxypteridine. In the normal strain, isoxanthopterin was identified in the chromatograms of the posterior region of the fatbody, but it was not obtained from the anterior region of the fatbody. On the other hand, xanthine dehydrogenase activity could be demonstrated throughout the fatbody of the normal strain. The restriction of isoxanthopterin to a certain group of fat cells in the wild type strain and its absence from other fat cells can be explained by the differential distribution of its immediate precursor, 2-amino-4-hydroxypteridine, as displayed in the mutant *rosy*.

Chromatographic separation of fluorescent substances from *Drosophila* has revealed quantitative as well as qualitative differences between the various mutant strains examined (10, 12). A number of these fluorescent compounds are pteridines, and their distribution in mutant strains is related to the phenotypic expression of eye color (3–5, 8, 11, 14, 30). Variations in the pattern of fluorescent compounds can be detected in individual organs and tissues of *Drosophila* by paper chromatography. Since the elaboration of these fluorescent substances is regulated by genetic mechanisms within the cells of these organs, the primary site of their occurrence must be correlated with the structural components of the cells themselves. Fluorescence microscopy should be useful in the detection of the autofluorescence of these structures within the cells and the distribution of this autofluorescence should correspond to the pattern of fluorescent compounds isolated by paper chromatography. These requirements have been realized in one instance. The cells of the anterior region of the larval fatbody of *D. melanogaster* accumulate light blue autofluorescent globules around the time of puparium formation (24). A single light blue fluorescent spot appears on paper chromatograms of this same region of the fatbody, and this fluorescent material has been identified

as kynurenine. Furthermore, this light blue fluorescent material does not appear in the anterior larval fatbody of the *vermilion* mutant which lacks kynurenine.

During the survey on the accumulation of kynurenine in the fat cells of various mutant strains, another type of fluorescent granule was noted in the fatbody of the mutant *rosy* (*ry*). The present report summarizes the distribution and character of the autofluorescent cytoplasmic content of the fat cells of the *ry* mutant. This mutant, whose biochemical pattern of deviation is identical to that of the mutants *rosy*^{2} (*ry*^{2}) and *maroon-like* (*ma-l*), is particularly interesting since a portion of its biochemical abnormality can be ascribed to the absence of the enzyme xanthine dehydrogenase (2, 6, 7, 9, 13, 15, 16, 18). As a result, *ry* flies accumulate the substrates for this enzyme, 2-amino-4-hydroxypteridine and hypoxanthine, and lack the resulting products of enzyme activity, isoxanthopterin and uric acid respectively. In addition, larger amounts of sepiapteridine and biopterin are found in *ry* flies than in normal flies.

METHODS

The methods of raising and collecting larvae and pupae for the examination were the same as those reported previously (23). The ages of larvae and pupae were timed from emergence from the egg and larval collections were made at 2-hour intervals. The wild type flies used were from the *Ore-R* strain. Preliminary observations were made with material from both the *rosy* and *rosy*^{2} mutants which are allelic and located on the third chromosome (1, 13) as well as with the *maroon-like* mutant, a sex-linked mutant (1). The detailed studies reported in this paper were confined to the *rosy* stock.

Tissues were dissected in freshly prepared Ringer solution. Some examinations were made in Tris buffer (0.1 M at pH 8.0) since the latter was used in preparations for enzyme activity. A definite leaching of fluorescent material was apparent when cells were placed in the buffer, but it tended to stabilize the yellow autofluorescent granular material, thus proving valuable for photography of these inclusions. Both one- and two-dimensional chromatograms of fatbodies from male specimens have been prepared. Quantitative differences in fluorescent substances between males and females have been reported (11, 12). Although no difference in fluorescence was noted between male and female fatbodies in the cytological examinations, it was decided to limit chromatographic analysis to male specimens of the same age in order to avoid any discrepancies of a quantitative nature that might result by combining both sexes. The solvent systems used by previous authors (6, 12, 27) have been utilized: isopropanol:1 per cent ammonia (2:1); butanol:acetic acid:water (4:1:1 and 4:1:5). In some instances, tissues were placed directly on the chromatographic paper and some tissue extracts after maceration have also been used. All chromatographic procedures were done in a dark room and dissections of the tissues for chromatographic analysis were performed in a dark room using a red filter in front of the microscope lamp.

A Zeiss fluorescence microscope equipped with a dark field condenser and mercury burner HBO-200 has been used; the filter system yielded principal excitation at 365 mμ and barrier filter GG4 has been used for all photography. Tissues have been placed directly on a Corning # CS 5860 filter (1.3 mm in thickness) to remove any visible light beyond 400 mμ which may have been generated in the optics of the substage condenser. Although desirable for critical analysis, this type of observation was inconvenient, since the same cell preparation could not be examined with visible light other than by epillumination Therefore, quartz and glass slides have also been used.

RESULTS

The cells of the anterior region of the larval fatbody of the *ry* strain contain light blue fluorescent globules similar to those described previously for the normal and other mutant strains of *Drosophila* (24). The extent and time of appearance of this autofluorescence due to kynurenine in the *ry* strain coincides with the normal pattern. In *ry* young prepupae the fat cells immediately posterior to this region of the fatbody and extending posteriorly to include the caudal fat masses contain bright yellow fluorescent granules. This type of yellow fluorescent granule in fat cells is not found in wild type prepupae of this age nor in prepupae of the following mutant strains: *cinnabar*; *cinnabar, sepia*; *sepia*; *maroon*; *red malpighian tubes*; *vermilion*; *scarlet*; *claret*; *white*; and *brown*. Since the mutants *ry*^{2} and *ma-l* are identical in their biochemical pattern of abnormality to the *ry* mutant, prepupae of these strains were also examined. In both cases bright yellow fluorescent granules were found in the posterior fat cells. No further examinations were made on the *ry*^{2} and *ma-l* stocks, other than to verify this pattern of similarity since *ma-l* shows a maternal effect, and the viability of the *ry*^{2} stock was lower than that of the *ry* stock.

The larval fatbody of *Drosophila* is a continuous tissue mass. Shortly after the pupal molt, the cells of the fatbody become separated from each other and are redistributed throughout the body of the pupa. When the body of the fully formed imago is opened, the fat cells float out freely. The development of the yellow fluorescent granules is most striking just prior to the dissociation of the cells of the fatbody when the air bubble in the abdomen is prominent. This stage has been described by Robertson (25) and will be referred to as the bubble stage. Buoyancy changes as the size of the bubble increases, and bubble prepupae can be classified as those which sink in Ringer solution or those which float. The former is the most desirable stage for examination of *ry* fat. The yellow fluorescent granules are irregular in shape. After brief exposure to ultraviolet light, the yellow fluorescence fades and the granules appear light cream-white. When examined with visible light, both brightfield and darkfield illumination, they are colorless refractile objects easily distinguishable from the other cytoplasmic inclusions found in the fat cells. If sufficient pressure is applied to the coverglass so that the cytoplasmic contents are allowed to escape from the cells, the nature of these inclusions can be examined more closely. Groups of these granules are found inside globular inclusions which can be ruptured by pressure to permit the release of the smaller granules. At the bubble stage each cell may contain from 20 to 32 of these globular packets of yellow fluorescent granules. The size and shape of the packets is variable.

In addition to the yellow autofluorescence, light cobalt blue fluorescent globules are found in the fat cells of the same region of *ry* prepupae. The latter type of globule is most readily detected when the cells are ruptured by applying pressure to the coverglass and the cytoplasmic contents are released into the surrounding medium. The cobalt blue fluorescent globules are larger than the individual yellow autofluorescent granules and are normally spherical unless distorted by pressure on the coverglass. The cobalt blue fluorescent globules in the posterior fat cells are less variable in size than the kynurenine globules in the anterior fat cells. Both the yellow autofluorescence and the cobalt blue fluorescence are confined to the posterior fatbody of *ry* and occur in the same cells. Fig. 7 provides a diagrammatic representation of the fatbody illustrating the extent of the various types of autofluorescent substances. The differences in cytoplasmic content of the anterior and posterior regions of the fatbody of the *Ore-R* wild type strain and the *ry* mutant are demonstrated in Figs. 1 to 6.

The yellow fluorescent granules are not found in the posterior fat cells of the mid third instar *ry* larvae, and the cytoplasm shows a diffuse light cobalt blue fluorescence. No large cobalt blue fluorescent globules similar to those described for the bubble stage are discernible in the cytoplasm. If the posterior fatbody of these larvae is placed in Tris buffer instead of Ringer solution, the cells lose their typical elasticity and texture. When these cells are then ruptured by pressure, cobalt blue fluorescent material is released from the cells in the form of globules of variable size. The nature of the cobalt blue fluorescent material at this stage is thus modified by the conditions employed in viewing it; in the prepupal stage, however, globular material in the cells is readily apparent in either medium.

For chromatographic analysis, the fatbodies of *ry* prepupae (bubble stage) were divided into anterior and posterior regions. The anterior region has been designated as that area in which kynurenine accumulates and the posterior region includes the remaining fat cells. Sufficient tissue to either side of the boundary region has been removed and discarded during the dissections to assure complete isolation of substances limited to either the posterior or anterior sections. For the samples of anterior region this procedure generally required retaining only fatbody anterior to the lateral commissure. The dissected tissues were rinsed in three changes of Ringer solution, and cellular debris together with any loose fat cells was removed during this procedure before the tissues were applied to the chromatographic paper. After removal from the solvents and drying, the chromatograms were examined with the ultraviolet light source and filter system which had been used for the microscopic observations.

The chromatograms of the anterior fatbody of *ry* prepupae showed a light blue fluorescent spot corresponding in Rf to the known sample of kynurenine. These results confirm previous chromatographic analysis of the anterior fatbody of *Drosophila* (24). A cobalt blue spot appeared only in the chromatograms of the posterior region of the fatbody, and the Rf of this spot was the

same as that of the known sample of 2-amino-4-hydroxypteridine (AHP) in both one- and two-dimensional chromatograms (Fig. 7). For a series of two-dimensional chromatograms, the second solvent was five per cent acetic acid following the propanol-ammonia solvent for the first dimension. Hubby and Forrest (16) used this method to separate biopterin from AHP. This procedure yielded a single blue fluorescent spot corresponding in Rf to AHP from the posterior fatbody of *ry* prepupae. Both the anterior and posterior tissues of the fatbody of *ry* pupae yielded faint yellow spots on the chromatograms, and no clear cut separation of a yellow fluorescent spot limited to the posterior fatbody was obtained. Experimentation with other solvent systems as well as with larger quantities of tissues must be planned for separation of the yellow fluorescent materials in the fatbody. In the present study, a single posterior fatbody of *ry* was sufficient to yield a detectable spot of AHP. Chromatograms with various quantities of anterior and posterior fatbodies ranging from 1 to 35 have been used.

The separation of Fl 3 from the fatbody of normal *Drosophila* larvae by Hadorn and Mitchell (12) and the later identification of this fluorescent material as isoxanthopterin raises an interesting question in relation to the differential distribution of AHP in the *ry* fatbody. Since the substrate AHP is accumulating in the posterior

Explanation of Figures

For all photographs in this plate: specimens were mounted on slides made of Corning #CS 5860 glass; microscope with a dark field condenser, 100× oil objective and 6× ocular; light source was mercury burner HBO-200, principal excitation 365 mμ with barrier filter GG4.

FIGURE 1

Photomicrograph of fluorescent globules in the anterior fat cells of a bubble stage prepupa (*Ore-R* normal strain) containing kynurenine. × 600.

FIGURE 2

The posterior fat cells from the same prepupa as Fig. 1. The light scattered from neighboring fluorescent structures is reflecting over the fat globules near the right margin of the photograph. × 600.

FIGURE 3

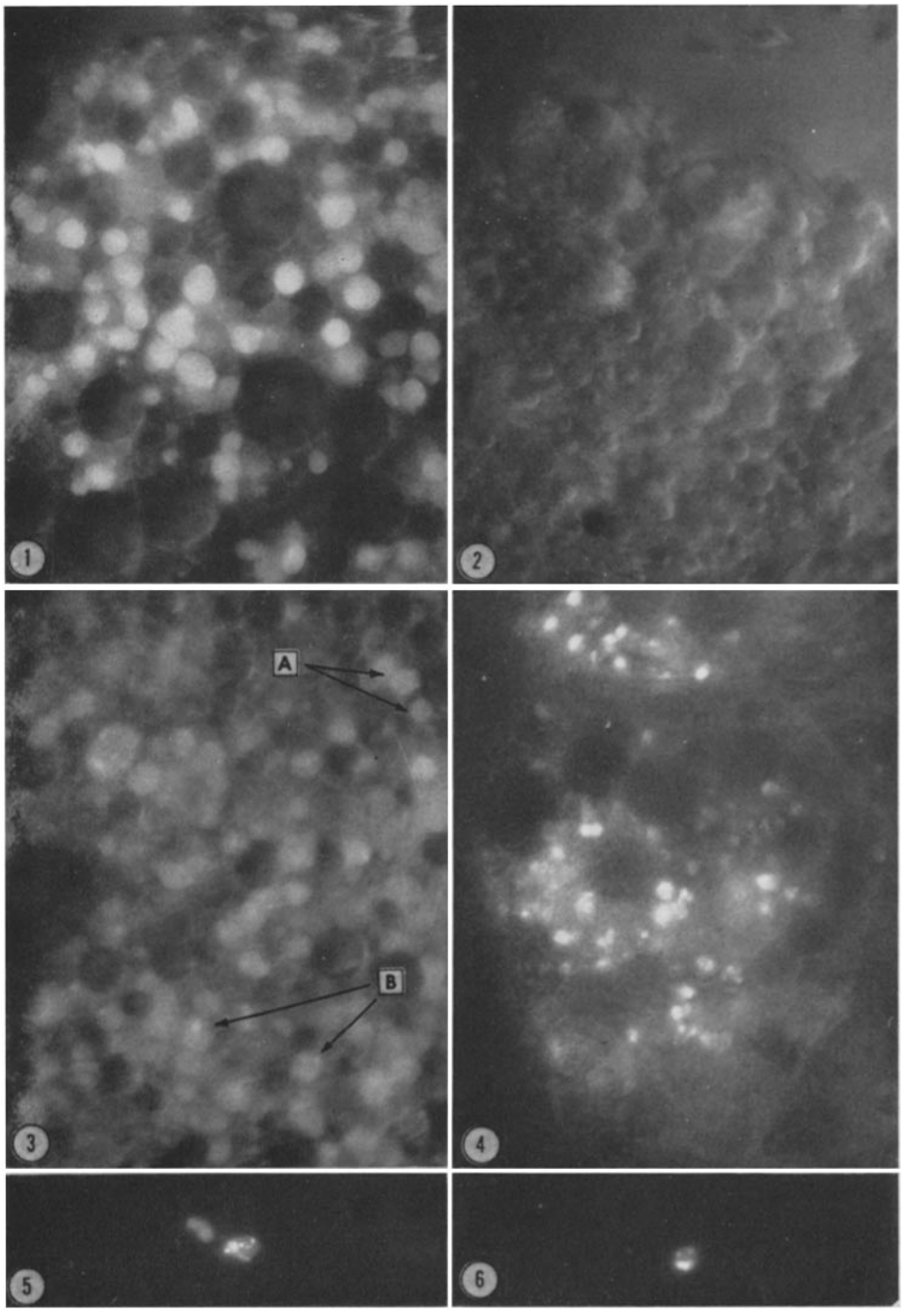
The fluorescent globules in the posterior fat cells from the early bubble stage prepupa of the *ry* mutant. There are two types of fluorescent inclusions: *A*, those which fluoresce cobalt blue and *B*, globules which contain the small yellow autofluorescent granules. × 600.

FIGURE 4

Later stage in the development of the autofluorescent substances in the posterior fat of the *ry* prepupa. The increase in number and brightness of the yellow autofluorescence tends to mask the cobalt blue fluorescence in photographs of this stage; the cobalt blue fluorescent globules in this stage are best viewed by flattening the preparation. × 600.

FIGURES 5 AND 6

Globules isolated from the posterior fat of the *ry* prepupa in 0.1 M Tris buffer at pH 8 showing the organization of the globules containing yellow autofluorescent granules. The internal illumination of the globule is due to the scattering of the fluorescence from the small yellow fluorescent granules. The number of granules within a globule and the shape of the globules is variable. × 600.



region of the fatbody of the *ry* pupae which lack xanthine dehydrogenase, will normal pupae which contain this enzyme show a similar pattern of distribution of the product mediated by the activity of this enzyme, isoxanthopterin? In order to examine this question, the fatbodies of *Ore-R*

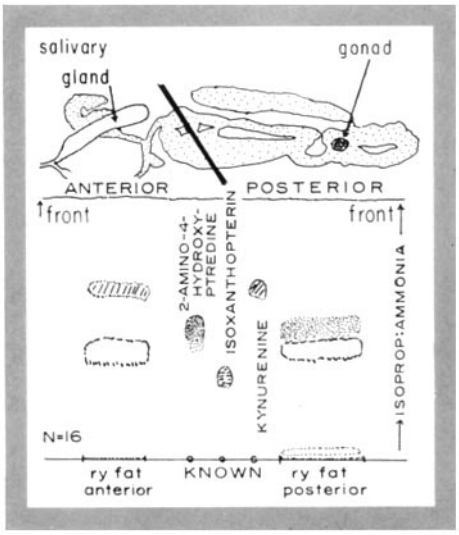


FIGURE 7

A tracing of a one-dimensional chromatogram to illustrate the differential distribution of fluorescent substances from the anterior and posterior regions of *ry* fatbodies isolated from bubble stage prepupae. Kynurenine is restricted to the anterior fatbody and 2-amino-4-hydroxypteridine is obtained only in the sample of posterior fatbodies. No spot corresponding in Rf to isoxanthopterin is found in the fatbody of the *ry* mutant. The identity of the other fluorescent spots on the chromatogram has not been established. A diagrammatic representation of a lateral half of the larval fat mass is included above the chromatogram to illustrate the extent of the areas designated as anterior and posterior. These regions have been divided by a cross bar. Number of specimens = N. Solvent system = isopropanol:1 per cent ammonia (2:1). Chromatographic paper, Whatman No. 1.

male prepupae were divided into anterior and posterior regions in the same manner as the groups of *ry* specimens. However, in this case the testes were removed from the fatbodies of the *Ore-R* pupae and chromatographed separately in order to establish their content of fluorescent substances at this stage. Hadorn (11) reported the presence of isoxanthopterin in the testes and noted a higher quantity of this substance in males than in females.

Chromatograms of the anterior fatbodies showed a fluorescent spot corresponding in Rf to kynurenine, while the chromatograms of the posterior fatbodies of *Ore-R* pupae yielded a purple fluorescent spot corresponding in Rf to the known sample of isoxanthopterin. In addition to the isoxanthopterin in the posterior fatbodies, a faint spot corresponding in Rf to AHP was also present. No blue or purple fluorescent spots appeared in the chromatograms of the testes of this age. Isoxanthopterin could be detected from half of the posterior fatbody of a single specimen of *Ore-R*; as many as 35 anterior fatbodies did not yield a detectable spot of isoxanthopterin.

The confinement of the precursor and its immediate product to a group of cells indicates that the cellular distribution of the enzyme must coincide with this pattern of distribution. However, will its coextensive distribution be such that the enzyme activity will be limited to the cells of the posterior region of the fatbody? The methods used by Forrest (2), Glassman (6), and Nawa (19) were adapted to an examination of the xanthine dehydrogenase activity in the fatbody of the normal *Ore-R* strain.

The anterior and posterior regions of the fatbodies of 30 *Ore-R* prepupae were separated and washed in Ringer solution. Each group was then transferred to 0.1 ml of 0.1 M Tris buffer at pH 8. The tissues were macerated and incubated with 0.02 ml of 10^{-3} M AHP and 0.02 ml of 10^{-2} M diphosphopyridine nucleotide (DPN) at room temperature. Control series, that is, all components minus the tissue extract, were maintained for all of the enzyme studies, and these were negative throughout these studies as well as those presented below. Control studies using boiled tissue extracts were also completed. An initial sample of 5 λ was applied as a streak to chromatographic paper and subsequent samples were similarly added after 1, 2, 4, and 8 hours. The chromatograms were run in isopropanol: 1 per cent ammonia (2:1) for 2 hours and examined with ultraviolet light after they were dried. A purple fluorescent streak corresponding in Rf to isoxanthopterin was present in all chromatograms of the posterior regions of the fatbody of *Ore-R* prepupae; this represented endogenous isoxanthopterin as well as that formed from the exogenous AHP, since there was a noticeable decrease in fluorescence of the AHP streak in samples taken after 2 hours of incubation and a correlated increase in isoxanthopterin.

This trend increased with the duration of incubation. The incubation mixture containing buffer, DPN, and AHP with anterior fat masses did not yield isoxanthopterin in the initial sample nor after 2 hours of incubation. After 4 hours of incubation, chromatograms prepared from the mixture containing anterior fat masses showed a faint indication of isoxanthopterin, and by 8 hours a well defined fluorescent streak was apparent. However, there was no perceptible decrease in AHP based on visual observation such as that evident with the posterior fat masses. Incubation of AHP with xanthine oxidase (Worthington Biochemical Corp., Harrison, N. J.) yielded isoxanthopterin under the conditions employed by Forrest *et al.* (2).

Samples of anterior and posterior fatbodies from 30 *Ore-R* prepupae were macerated in Tris buffer followed by Norit-A charcoal treatment as used by Glassman (6, 7). After charcoal treatment, the mixture was centrifuged at 3000 RPM and the clear supernatant was used as the source of enzyme. Chromatograms were prepared as previously after incubation with AHP and DPN. The charcoal treatment removed endogenous isoxanthopterin from the sample of the posterior fatbodies, and isoxanthopterin was produced after incubation with AHP. No isoxanthopterin was detectable in any of the chromatograms of the anterior fatbodies by this method even after 8 hours of incubation with AHP. These results with and without charcoal treatment proved consistently reproducible in four series of experiments. A review of the diagram (Fig. 7) illustrates the difference in size of the area which has been designated anterior fatbody and that termed the posterior region. As mentioned above, for all dissections the tissue in the boundary region has been discarded to assure complete separation of anterior and posterior regions. This procedure requires exclusion of a considerable portion of the anterior region which initially is much smaller than the posterior region. Any experiments comparing these two regions must, therefore, take account of this quantitative discrepancy of tissue mass. If the assumption is made that the tissue of the posterior region used in the enzyme studies is five times the bulk of the anterior region used, then posterior fatbodies from six specimens should comprise a quantity equal to anterior fatbodies from 30 specimens. The experiment utilizing charcoal treatment was, therefore, repeated on a sample of six posterior fatbodies, and in this case, no isoxanthopterin could be detected after incubation with AHP and DPN. These experiments indicate a decrease in the enzyme activity after charcoal treatment and this decrease is sufficient to deplete below detection the activity of six posterior fatbodies. The negative result after charcoal treatment of 30 anterior fatbodies may thus be explained on the basis of tissue mass rather than a difference in distribution of enzyme activity between the two regions of the fatbody.

DISCUSSION

The larval fatbody of *D. melanogaster* is a continuous tissue mass composed of a morphologically homogeneous cell population. The cytoplasm of these fat cells consists of many globular inclusions but characteristics based on the usual cytological techniques of staining picture a morphological similarity of the cells throughout the fatbody. In view of this apparent morphological similarity in the normal strain, the mutations which effect regional alterations within the fatbody are particularly intriguing. An example of a mutant factor which specifically influences one region of the fatbody is the *tumor*^{w} (*tu*^{w}) factor (21, 22, 32). Melanization occurs in the caudal region of the fatbody of the *tu*^{w} strain, and through selection, high penetrance and regional specificity of melanization have been attained in some lines. The abnormal pattern in *tu*^{w} involves a specific encapsulation of the fat cells of the caudal region by one class of blood cells and subsequent melanization of this encapsulated mass. Either the site of these fat cells with relation to other factors in the body, or some alteration within the fat cells of the caudal region distinguishes them from the other cells of the fatbody in the *tu*^{w} strain so that only the former are consistently involved in the formation of abnormal melanotic masses. A similar example is provided by the *red cell* (*rc*) mutant (17) and the mutant combination *tu*^{w}*rc* (23) in which the cells of the anteriormost region of the fatbody develop pigment after a period of larval starvation. Examination of the fatbody of the *rc*, *tu*^{w}*rc*, and the wild type strains with the fluorescence microscope revealed that at the time of puparium formation the cells of the anterior fatbody of all of these strains accumulate light blue fluorescent globules (24). This fluorescent material has been identified as kynurenine, a precursor of the brown eye pigment of *Drosoph-*

ila. The extent of the kynurenine accumulation in the larval fatbody coincides with that region of the fatbody which develops pigment in the starved *tu*^{w}*rc* mutant combination. Detection of regional differentiation in the fatbody of the wild type strain is thus possible by fluorescence microscopy; the inherent biochemical differences between cells of the various regions of the wild type fatbody serve to explain how mutant characteristics may be restricted to a particular section of this tissue mass. A similar differentiation of cells within the liver lobules of the rat has been reported recently on the basis of enzyme activities and patterns of morphological characteristics of the mitochondria (20, 26). These differences are a reflection of the location of the cells within the lobules, the peripheral cells differing from those in the central portion of the lobule.

The deficiency of xanthine dehydrogenase in the *ry* mutant is responsible for the absence of isoxanthopterin and excessive accumulation of its precursor, AHP (6, 13). An increased quantity of hypoxanthine combined with the lack of uric acid in *ry* flies can also be explained by the absence of this enzyme activity (18). This same pattern of abnormality is found in the *ry*, *ry*^{2}, and the *ma-l* mutants (6, 9, 16, 18). A study of the fatbody of the *ry* mutant was prompted by the presence of fluorescent granules in the posterior region, and the confirmation that similar yellow autofluorescent material appeared in the *ry*^{2} and *ma-l* mutants as well. There is a mutual exclusion such that the fat cells which accumulate large globular inclusions containing kynurenine do not possess the yellow autofluorescent granules in the *ry* strain. The latter appear in what might be termed the mid and posterior regions of the larval fatbody, and these cells with the yellow fluorescent granules also contain AHP in the *ry* mutant. Identification of the intracellular site of both AHP and kynurenine is based on the comparison of the color of autofluorescence *in situ* with chromatographic separation from these same cells of a fluorescent spot of identical color having the same Rf as a sample of the pure substance. This procedure, of course, is feasible if only one spot on the chromatograms compares in fluorescence with the cytological autofluorescence, and there is complete agreement regarding simultaneous detection by both methods. The spherical, globular nature of the inclusions with AHP differs from the irregular, granular appearance of the yellow autofluorescent inclusions. Until the chemical identity of the latter is established, the relationship between this material and AHP in the same cells remains hypothetical. The yellow autofluorescent granules have not been noted in the wild type fatbody at this developmental stage nor in the other mutant strains examined, with the exception of the *ry* alleles and *ma-l*. The excessive accumulation of AHP and the appearance of the yellow autofluorescent granules in the same cells within this group of related mutants suggest pleiotropy at the intracellular level. Detection of AHP in the fat cells of *ry* precedes the appearance of the yellow autofluorescent granules. The larval fatbody of the normal strain contains isoxanthopterin at this age when the *ry* fatbody has easily detectable amounts of AHP. The development of the yellow autofluorescence in the *ry* strain may be initiated only after a considerable pile-up of AHP due to the block in the step leading from AHP to isoxanthopterin. In a schema such as that outlined by Taira (28, 29) linking the red eye pigments and isoxanthopterin, such an accumulation of another precursor related to sepiapteridine would be reasonable. On the other hand, the yellow autofluorescent substance may be indicative of the upset in uric acid metabolism in the *ry* mutant, since it is known that in many insects uric acid and urates appear in fat cells during certain stages of development (review in Wigglesworth, 31). Another possibility may also be considered. The yellow autofluorescent granules may be the product of more than one deviation with the yellow fluorescent material depositing on the small granules as they accumulate.

The region of the fatbody which accumulates AHP in the *ry* mutant corresponds with that region in the normal strain which develops isoxanthopterin. The differential distribution of isoxanthopterin in the normal larval fatbody at this stage of development is interesting considering its wider distribution during later development (14, 15). The testes contain a considerable quantity of this substance in the adult (11, 12), but at the time when the posterior larval fatbody contained isoxanthopterin, none was detected in the testes in the present study. The larval testes are embedded in the posterior fatbody, and the relationship between these organs with regard to the accumulation of isoxanthopterin as pupal life progresses should be examined. The non-autonomous nature of the

appearance of isoxanthopterin after implantation of eye disks (14) implies diffusibility of substances at some point in the biochemical pathway leading to this metabolite, or induction of the necessary prerequisites by an external stimulus. With the use of C^{14}-labeled AHP, Hubby and Forrest (16) found that this precursor is converted *in vivo* exclusively to isoxanthopterin and not into any of the other pteridines or red pigments of *Drosophila*. The regional restriction of AHP within the fatbody of *ry* explains the differential pattern of isoxanthopterin between the regions of the fatbody of the normal strain. The activity attributed to xanthine dehydrogenase was found throughout the fatbody, so in this instance, substrate localization is responsible for the distribution pattern of a specific metabolite within a group of cells.

The mutants of *Drosophila melanogaster* used in this study were kindly provided by Dr. E. B. Lewis from the stock collection at the California Institute of Technology. We are grateful to Dr. H. S. Forrest who has given us generous samples of isoxanthopterin and 2-amino-4-hydroxypteridine for use in the chromatographic analyses.

This paper is dedicated to Professor L. C. Dunn in recognition of his long and distinguished career.

This study was supported by Research Grant RG-5285 from the National Institute of Health, Public Health Service, while the authors were at Reed College, Portland, Oregon.

Received for publication, July 2, 1961.

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