AN INDUCIBLE ENZYME SYSTEM IN THE LARVAL CELLS OF *DROSOPHILA MELANOGASTER*

T. M. RIZKI, Ph.D., and ROSE M. RIZKI

From the Department of Zoology, The University of Michigan, Ann Arbor

ABSTRACT

An adaptive increase in tryptophan pyrrolase activity was obtained in the cell-free extracts of *Drosophila melanogaster* larvae which were given a dietary supplement of L-tryptophan. This activity was detectable in the extracts prepared from larval fatbodies when these were isolated from the remaining body tissues, but the present methods did not reveal any activity in extracts from the latter. These experiments confirm previous observations on the cellular distribution of kynurenine after feeding experiments with tryptophan. The autofluorescence characteristic of kynurenine in the larval fatbody is limited to the cells of the anterior region under normal feeding conditions, but, when larvae are fed tryptophan, autofluorescent kynurenine globules are found in a larger number of fat cells. *In vitro* incubation of isolated posterior fat cells with tryptophan has shown that these cells are capable of producing kynurenine. It is this same region of the fatbody in the *suppressor of vermilion* mutant that develops kynurenine autofluorescence, thus indicating that the absence of kynurenine in these cells in the normal strain is the result of an inhibition of their genetic potential to produce kynurenine. It has been concluded that the differentiation of "kynurenine cells" in the fatbody is controlled by a genetic mechanism which operates through the inducible tryptophan pyrrolase system.

The cells of the anterior region of the larval fatbody of *Drosophila melanogaster* accumulate kynurenine toward the end of larval life. This regional localization of kynurenine synthesis is maintained when the larvae are fed yeast under the usual culture conditions or if the larvae are submitted to a brief period of starvation. Addition of tryptophan to the diet of third instar larvae increases kynurenine production in the fatbody with the result that a greater number of fat cells contain globular cytoplasmic inclusions displaying the characteristic light blue autofluorescence of kynurenine (12, 13).

The inducible nature of tryptophan pyrrolase in mammalian liver has been established by Knox (6, 8). Glassman reported the presence of kynurenine formamidase in *Drosophila* (4), and recently tryptophan pyrrolase activity has also been demonstrated in *Drosophila* (2). The present report summarizes a study of tryptophan pyrrolase activity in the fatbody of *Drosophila melanogaster*, and relates this activity to the distribution of the autofluorescent pattern of the fat cells.

MATERIALS AND METHODS

The *Ore-R* wild type strain and the unsuppressible *vermilion* mutant, v^{36f}, have been used. The age of larval life has been recorded from occlusion, and the larvae have been raised on cream of wheat–molasses medium seeded with a yeast suspension. All experimental material has been maintained at 24°C in an incubator, and the dissection of tissues as well as the *in vitro* studies was performed at 23–24°C. At 65 hours

of age, larvae were flooded out of the medium with a saturated NaCl solution, rinsed briefly with a 1 per cent solution of NaOCl followed by six rinses with distilled water. Larvae were then transferred to dishes containing washed Whatman cellulose powder moistened with a 1 per cent L-tryptophan solution. Control groups of larvae were placed on cellulose powder with distilled water. In some experiments a yeast suspension was added to the dishes with tryptophan, and the control dishes for these experiments consisted of cellulose powder moistened with yeast suspension.

Cell-free extracts of the larvae were prepared when the larvae were 90 to 92 hours of age. The method presented by Knox (7) was used for the studies of tryptophan pyrrolase activity, modifications including the addition of phenylthiourea and the adjustment of volumes so that incubation mixtures between 0.25 ml and 0.33 ml were used. Phenylthiourea (0.2 mg/ml KCl-KOH solution) was added to the homogenizing tubes to inhibit tyrosinase activity which is pronounced in larvae approaching pupation. Tryptophan pyrrolase activity was not detectable unless tyrosinase activity, apparent by the blackening of the incubation mixture, was inhibited. Glassman (5) has demonstrated that products of tyrosinase activity such as *o*-quinones can combine with kynurenine to form pigments, and such a phenomenon might account for the interference with detection of kynurenine in the incubation mixtures without phenylthiourea. The presence of phenylthiourea would also serve to remove Cu^{++} which Knox has noted is an inhibitor of tryptophan pyrrolase activity. Cu^{++} is present in the midgut and Malpighian tubules of *Drosophila* larvae (11), and would therefore be contained in the homogenates of total

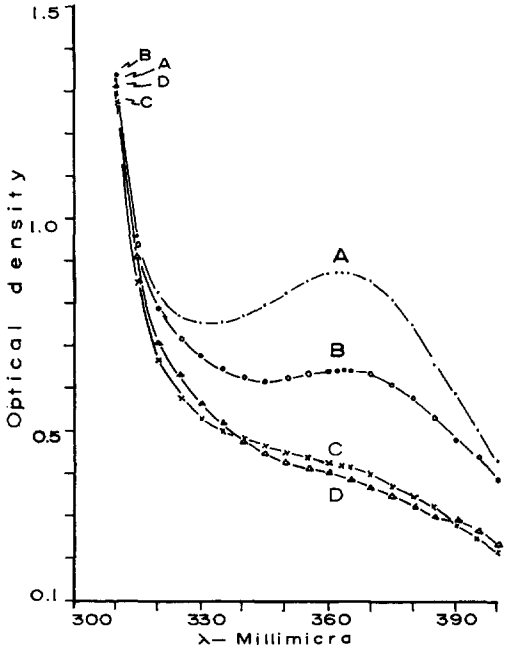

FIGURE 1

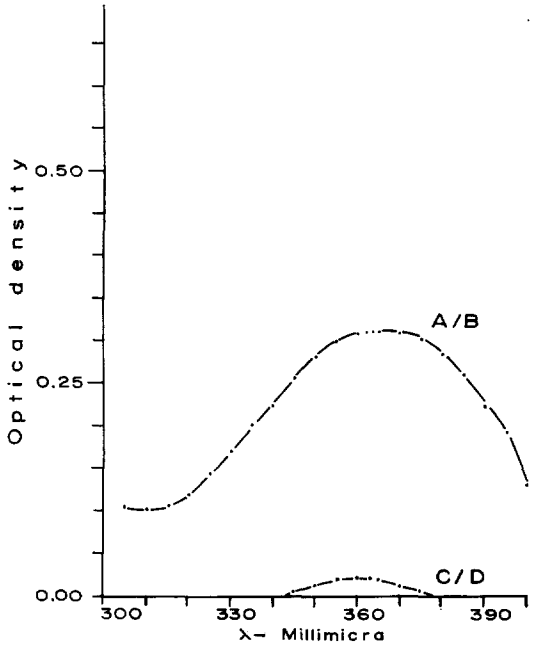

FIGURE 2

FIGURES 1 AND 2

An example of the adaptive increase in tryptophan pyrrolase activity is indicated by the differences in the absorption curves of the kynurenine produced by larvae fed on tryptophan (A and B) and larvae placed on H_2O (C and D). Fig. 1 shows the absorption curves of the incubation mixtures using H_2O in the reference cuvette. A and C present the activity of the cell-free extracts when incubated with L-tryptophan, and B and D are the control extracts incubated without substrate. In this experiment 100 tryptophan-fed larvae with a wet weight of 111.8 mg were used, and the 100 larvae on H_2O that were used weighed 108.5 mg. Each curve represents the amount of kynurenine per 50 larvae since the homogenate was equally divided between the two tubes, with and without substrate. Fig. 2 graphically presents the difference between the pyrrolase activity of the tryptophan-fed and unfed larvae by subtracting the absorption values of curve B from A (A/B) and of curve D from C (C/D).

larvae. Incubation with L-tryptophan in an O_2 atmosphere proceeded for 3 hours in a water bath shaker at 37°C. The control tubes contained all ingredients except tryptophan. Optical density readings between 300 mμ and 400 mμ were taken with a Zeiss PMQ II spectrophotometer.

For the *in vitro* observations the same L-tryptophan solution as prepared for the studies with cell-free extracts was used. In this case, however, the tryptophan solution (0.13 ml of 0.03 M L-tryptophan) was added to Ringer's solution (0.32 ml) and the tissues were incubated at 23°C in an O_2 atmosphere for 30 minutes. The control for these experiments consisted of incubating tissues in Ringer's solution. The autofluorescence of these tissues was then examined with a Zeiss fluorescence microscope.

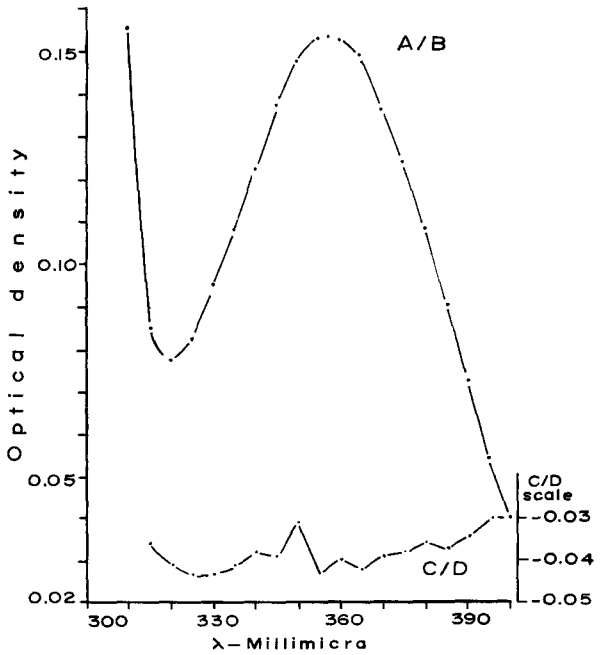

FIGURE 3

Absorption curves of kynurenine produced from the cell-free extract of fatbodies (A/B) of tryptophan-fed larvae and the extract of the remainder of the bodies (C/D) of these same larvae. 50 larvae were dissected in this experiment. Curve A/B presents the difference between the incubation mixtures of fatbodies with and without substrate; curve C/D is the difference between the incubation mixtures of the residual body extract with and without substrate.

RESULTS

An adaptive increase in tryptophan pyrrolase activity was obtained when *Drosophila* larvae were fed L-tryptophan. This increase as recorded in the absorption curves of kynurenine (Figs. 1 and 2) resulted when larvae were fed tryptophan from the midthird instar and the enzyme studies undertaken shortly before pupation. Less enzyme activity was obtained from larvae maintained on water from 65 hours of age than from larvae that were feeding on yeast. The values for the enzyme activities of larvae on water averaged 2.3 $\times$ 10^{-5} μmoles kynurenine/larva/hour; larvae feeding on yeast averaged 6.8 $\times$ 10^{-5} μmoles kynurenine/larva/hour; and larvae given tryptophan produced between 2.0 to 3.6 $\times$ 10^{-4} μmoles kynurenine/larva/hour.

In the attempts to localize enzyme activity all larvae were given tryptophan in order to raise the level of tryptophan pyrrolase. The fatbodies from tryptophan-fed larvae were separated from the other body tissues in Ringer's solution. The fatbodies and the remaining tissues from these larvae were then incubated separately in L-tryptophan. In different experimental runs, 40 to 50 larvae were used for each experiment and the homogenates were divided equally between the control tube lacking substrate and the incubation

tube with tryptophan. In each instance tryptophan pyrrolase activity was obtained from the fatbodies, but no activity was recorded in the homogenates from the other body tissues (Fig. 3).

Under normal feeding conditions, kynurenine in the fatbody is limited to the anterior cells and is not detected in the posterior cells. As described previously, "kynurenine cells" are in immediate contact with pteridine cells in the fatbody and diffusion of this metabolite from one region of the fatbody to another does not occur. A dietary supplement of tryptophan increases the quantity of kynurenine in the fatbody and examination with the fluorescence microscope reveals that the fluorescence characteristic of kynurenine is no longer limited to the anterior cells but is found in some posterior cells as well (12, 13). It may be questioned whether, under these experimental conditions, the anterior cells produce larger quantities of kynurenine which then diffuse to other fat cells. This question can only be answered by separating posterior fat cells from anterior fat cells and placing them in an environment with the inducer tryptophan. Since it has been demonstrated (13) that the development of kynurenine in the anterior fat cells is an autonomous process, a technique of *in vitro* incubation would provide the most convenient method to study the accumulation of kynurenine in isolated groups of cells. *In vitro* incubation was accomplished by placing the fat cells in Ringer's solution in watch glass Syracuse dishes standing in a chamber provided with an O_2 atmosphere. The control tissues were incubated in Ringer's solution for 30 minutes whereas the experimental tissues were incubated for the same length of time in Ringer's solution containing L-tryptophan. Under normal feeding conditions the fat cells immediately anterior to the gonad do not have kynurenine globules, but these same cells in larvae that have been fed tryptophan contain kynurenine globules. This region of the posterior fatbody was therefore selected for *in vitro* studies, and reference to posterior fat cells in the following discussion refers to this specific group of fat cells. After 30 minutes' incubation in Ringer's solution, the posterior cells showed the characteristic cytoplasmic fluorescence of isoxanthopterin. Rupture of the cells to observe the cytoplasmic globular contents did not reveal any globules with the characteristic light blue autofluorescence of kynurenine. After 30 minutes' incubation with L-tryptophan, however, the posterior fat cells contained light blue autofluorescent globules, and rupture of the cells released these globules which were then readily viewed as comparable in fluorescence to the kynurenine globules normally found in the anterior fat cells. These observations on the *in vitro* development of kynurenine in isolated posterior fat cells indicate that these cells possess the necessary requirements to convert tryptophan to kynurenine.

As an additional series of control experiments, the fat cells of v^{36f} larvae were used for *in vitro* studies. The v^{36f} mutant, which is genetically blocked in the conversion of tryptophan to formylkynurenine, does not show light blue autofluorescent globules in the anterior fat cells, and such autofluorescence characteristic of kynurenine was not obtained when v^{36f} larvae were fed L-tryptophan. These globules appeared in the fatbodies of v^{36f} larvae which were fed kynurenine from 65 hours of age until pupation. *In vitro* incubation of v^{36f} fatbody in tryptophan solution did not lead to the development of fluorescent kynurenine glolules. Fat cells from v^{36f} larvae, however, showed fluorescent kynurenine globules after 30 minutes incubation in Ringer's solution containing kynurenine. From these observations on the v^{36f} fat cells it may be concluded that the globular site for kynurenine accumulation is present in this mutant strain.

DISCUSSION

Kynurenine in the larval fatbody of *Drosophila melanogaster* is restricted to the cells in the anterior region whereas the remaining fat cells show an autofluorescence due to their pteridine content (12, 14). When *Drosophila* larvae are fed L-tryptophan, some of the cells, which under normal feeding conditions contain isoxanthopterin, will accumulate autofluorescent kynurenine globules. This extension of the autofluorescence due to kynurenine in the fatbody of tryptophan-fed larvae is viewed utilizing fluorescence microscopy and the increased quantity of kynurenine in the fatbodies of tryptophan-fed larvae has been verified by chromatography (13). The occurrence of tryptophan pyrrolase activity in the larval fatbody and its adaptive increase through the dietary administration of tryptophan therefore agree with the observations on the cellular accumulation of autofluorescent cytoplasmic globules containing kynurenine. The microscopic observations of the cellular autofluorescence are of particular value,

for in this instance they indicate that the increased quantity of kynurenine resulting from the inducer tryptophan involves the participation of cells that under normal feeding conditions are not characterized as "kynurenine cells." The accumulation of cytoplasmic kynurenine in the latter cells is a product of synthesis by these cells, for it has been demonstrated that this group of cells will produce kynurenine when incubated with L-tryptophan under *in vitro* conditions. From these observations it is significant that, during the adaptive enzyme increase, not only is the enzyme activity on the whole increasing, but the proportion of cells as units with this enzyme is also increasing. It is this latter demonstration which provides an insight into the role of inducible enzymes in the processes of differentiation.

The inducible nature of tryptophan pyrrolase has been established in mammalian liver (6, 8) and the factors influencing the induction of this activity have been investigated. Feigelson (3) has suggested that the induction of tryptophan pyrrolase by the inducer tryptophan involves the conversion of apoenzyme molecules to an active form of the enzyme, and Pitot (10) has indicated that the activation of this enzyme may be related to the release of a particulate form of the enzyme protein to a soluble state. Induction of tryptophan pyrrolase through the administration of tryptophan does not occur in embryonic liver, and tryptophan pyrrolase activity is not detected until after birth (1, 9). The experiments on the tryptophan pyrrolase activity of the fatbody of *D. melanogaster* have been limited to the late third larval instar and early puparium formation when the fat cells have attained their maximum size and when kynurenine globules are visible by fluorescence microscopy. A developmental sequence of the induction of this activity in the fatbody has not been completed, but it has been determined (unpublished data) that the increased number of "kynurenine cells" obtained by excess dietary tryptophan will not occur if larvae are fed tryptophan and then returned to a normal diet for a brief period before pupation. A high level of tryptophan in the diet must be maintained until the time the larvae leave the food and prepare to pupate. Furthermore, the accumulation of kynurenine within cytoplasmic globules in the fat cells does not appear to be influenced directly by hormonal factors associated with pupation. When larvae were ligated in midthird larval instar prior to the accumulation of kynurenine globules, the latter globules accumulated in anterior fat cells irrespective of whether these cells were located in the region of the body which had pupated containing ring gland or in the body section remaining larval due to the absence of ring gland.

The *vermilion* mutants of *D. melanogaster* are blocked in the conversion of tryptophan to formylkynurenine, and the fat cells in these strains do not show the characteristic light blue autofluorescence of kynurenine. *Vermilion* eye color is a result of the absence of the latter brown pigment precursor. Normal eye color is restored in the *vermilion* mutant when the genotype contains the mutant allele, *suppressor of vermilion*. The larval fatbody of the mutant combination of *vermilion* with its *suppressor* (*su*^{2}-*s v*) contains autofluorescent globules with kynurenine, and these globules are found in a larger number of fat cells than in those of wild-type larvae (13). The extension of "kynurenine cells" in the *su*^{2}-*s v* fatbody resembles the extension which is obtained in the normal strain by feeding tryptophan. However, the kynurenine autofluorescence of tryptophan-fed larvae is more intense and is found in more cells than in the suppressor pattern. It has been concluded that the differentiation of the normal fatbody into anterior "kynurenine cells" and posterior "isoxanthopterin cells" is the result of an inhibition (13), for the cells of the posterior fatbody which do not normally accumulate kynurenine have not lost the genetic potential to do so. This potential is not expressed in the presence of the normal allele of *su*^{2}-*s*, but the mutant allele at this locus allows the expression of this potential. A dietary supplement of L-tryptophan likewise overcomes this inhibition in the posterior fat cells and leads to kynurenine accumulation. Larvae which are fed tryptophan show an adaptive increase in tryptophan pyrrolase activity, and the extended degree of autofluorescence apparent in the fat cells is obtained under these conditions of increased enzyme activity. *In vitro* incubation of isolated posterior fat cells confirms that these cells can produce kynurenine when they are given the inducer tryptophan. The cellular differentiation of "kynurenine cells" is thus regulated by genetic mechanisms operating through an inducible enzyme system.

This investigation was supported by Research Grants RG-9203 and RG-9167 from the National Institutes of Health, Public Health Service.

Received for publication, October 19, 1962.

BIBLIOGRAPHY

1. AUERBACH, V. H., and WAISMAN, H. A., *J. Biol. Chem.*, 1959, **234,** 304.
2. BAGLIONI, C., *Heredity*, 1960, **15,** 87.
3. FEIGELSON, P., and GREENGARD, O., *J. Biol. Chem.*, 1961, **236,** 153.
4. GLASSMAN, E., *Genetics*, 1956, **41,** 566.
5. GLASSMAN, E., *Arch. Biochem. and Biophysics*, 1957, **67,** 74.
6. KNOX, W. E., *Brit. J. Exp. Path.*, 1951, **32,** 462.
7. KNOX, W. E., *in* Methods in Enzymology, (S. P. Colowick and N. O. Kaplan, editors), New York, Academic Press Inc., 1955, **2,** 242.
8. KNOX, W. E., *in* Synthesis of Molecular and Cellular Structure, 19th Growth Symposium, (D. Rudnick, editor), New York, Ronald Press Company, 1960, 13.
9. NEMETH, A. M., *J. Biol. Chem.*, 1959, **234,** 2921.
10. PITOT, H. C., AND CHO, Y. S., *Cold Spring Harbor Symp. Quant. Biol.*, 1961, **26,** 371.
11. POULSON, D. F., AND BOWEN, V. T., *Exp. Cell Research*, 1952, suppl. 2, 161.
12. RIZKI, T. M., *J. Biophysic. and Biochem. Cytol.*, 1961, **9,** 567.
13. RIZKI, T. M., *J. Cell Biol.*, 1963, **16,** 513.
14. RIZKI, T. M., and RIZKI, R. M., *J. Cell Biol.*, 1962, **12,** 149.