Organ Distribution of *Drosophila* L-3-Hydroxyacid: NAD Oxidase

L-3-hydroxyacid dehydrogenase (B-L-OHDH) (E.C. 1.1.1.45) is believed[1] in mammals to be involved in the glucuronate pathway whereby D-glucuronic acid is converted to D-xylose 5-phosphate. However, its role in the metabolism of *Drosophila* is currently unknown. BORACK and SOFER[2] have purified this enzyme from adult *Drosophila melanogaster*. They have shown it to be soluble and specific for the L-isomer only of gulonate and 3-hydroxybutyrate.

The current investigation is aimed at contributing to the elucidation of the metabolic role of the enzyme in *Drosophila* by determining its specific activity in the organs of larvae of *Drosophila melanogaster*.

Materials and methods. On successive days, approximately 50 third instar larvae of the strain Daekwanryeong were dissected in ice-cold 0.05 *M* phosphate buffer, pH 7.5. Each day a different organ was isolated and pooled in a 3 ml test tube containing a small amount of the dissection buffer, and assayed for total enzyme activity and soluble protein. Each pooled organ was washed 3 times in the homogenization buffer, hand homogenized with a teflon pestle and centrifuged at $17{,}000 \times g$ for 20 min. B-L-OHDH activity was determined in the supernatant using a Beckman Acta III double beam recording spectrophotometer. The cuvette chamber and assay solutions were maintained at 30 °C., and all assays were begun by adding enzyme. NADH production was measured by an increase in absorbance at 340 nm, and 1 unit of activity is defined as the production of 1 nmole of NADH per min. Enzyme activity was measured in an assay solution consisting of a final concentration of $3 \times 10\text{-}3$ *M* NAD, 0.04 *M* L-gulonate, 0.16 *M* pyrazole (inhibits *Drosophila* alcohol dehydrogenase[3]) in 0.1 *M* *tris*-HCl, pH 8.2, to a final volume of 1.1 ml. Protein was determined by the method of LOWRY et al.[4]

Results and discussion. The Table shows that L-3-hydroxyacid dehydrogenase (B-L-OHDH) has its greatest specific activity in the Malpighian tubules. It also shows that the enzyme is present inr elatively high activity in the Malpighian tubules and intestine. There are relatively small amounts of activity in the carcass, brain and fat body. The enzyme was not detectable in the salivary glands or imaginal discs. BORACK[5], using histochemical staining of the intact isolated organs of larvae of the same strain of *Drosophila melanogaster*, demonstrated a similar distribution of activity of this enzyme. In that study the brain did not show B-L-OHDH activity. Apparently in the brain, either the enzyme is present at a level which is too low to be detected histochemically, or this organ is impermeable to one or more of the components of the histochemical staining solution.

Organ	Enzyme units	Protein (mg)	Specific activity[a]
Malpighian tubule	72	0.347	207.5
Intestine	60	1.420	42.2
Carcass	9	1.055	8.5
Brain	4	0.570	7.0
Fat body	6	1.260	4.8
Salivary gland	not detectable	0.110	—
Imaginal disc	not detectable	0.235	—
10 whole larvae/larva	2.6	0.141	18.4

[a] Enzyme units/mg protein.

In the present investigation it was not possible to separate the chitinous carcass from the attached skeletal muscles, and the specific activity of the 'carcass' includes both these strucures. Histochemically[5] the skeletal muscles showed intense staining for the enzyme, while the chitinous carcass showed none. Presumably the designated activity of the 'carcass' in the present report is contained within the attached skeletal muscles. There are no other reports in the literature of the organ or tissue distribution of B-L-OHDH in invertebrates. However, the enzyme has been shown in hog and beef[6] and sheep[7] to be present in the highest activity in the kidney. The mammalian kidney is considered to be analogous to the Malpighian tubules of *Drosophila*. KOUNDAKJIAN and SNOSWELL[7] determined the tissue distribution of 3-hydroxybutyrate dehydrogenase in the cytosol of sheep. Subsequently[8] they showed that this activity is that of B-L-OHDH. Assuming that the activity of the carcass in the present investigation is that of the skeletal muscles, then the tissue distribution of the enzyme in sheep[7], determined at μmol/h per g of tissue, is identical in order of distribution to that of the specific activity of the enzyme in the analogous organs in *Drosophila*. This order of distribution of B-L-OHDH activity within the organs of *Drosophila* larvae is different than that of alcohol dehydrogenase[9] and soluble aconitase and NADP-isocitrate dehydrogenase[10]. The current investigation suggests a similarity of function of B-L-OHDH in both mammals and *Drosophila* based on distribution of the enzyme within the organisms. Further elucidation of the role of B-L-OHDH in *Drosophila* may be forthcoming in studies which are currently in progress[11].

Résumé. L'activité spécifique de L-3-hydroxy acide déhydrogénase a été déterminée dans les organes isolés de la larve de *Drosophila malanogaster*. L'enzyme présente son activité spécifique la plus intense dans les tubules de Malpighi et de moins en moins marquée dans la cuticule, le cerveau et le corps gras.

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