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ALDOLASE C IN RAT FETAL LIVER

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SUMMARY

The presence of aldolase C in rat fetal liver is a controversial problem which seems to stem from the methods used to study it as well as the cellular composition of the liver.

It is discussed in the light of new data obtained from enzymatic, electrophoretic and immunological methods.

Two types of aldolases (EC 4.1.2. and 4.1.2.13) were known (aldolase A, preponderant in muscle and aldolase B, preponderant in liver), when the third type was discovered in 1966 by Penhoet et al. [1] and Christen et al. [2]. Aldolase C from brain is more acidic than aldolase A and is easily resolved into five bands by electrophoresis [3]. This result may be explained by a tetrameric model able to produce two pure tetramers: (c_4 and a_4), and three hybrids. This structure was confirmed by differents workers [4–6]. The three types of aldolase react with fructose 1-6-diphosphate (Fru-1,6-P_2) and fructose 1-phosphate (Fru-1-*P*) at different rates. So the activity ratio Fru-1,6-P_2/Fru-1-*P* is 50 for aldolase A, 1 for aldolase B, 5–10 for aldolase C according to the species.

At first, aldolase C appeared to be localized selectively in the nervous mammalian tissues. But later, it was discovered in embryo and adult birds [7–9], in several mammalian tissues [10–20] and even in invertebrates [21].

Ontogenic studies concerning the three aldolases were performed in various species. The results are controversial especially concerning rat liver. Different methods were used by different authors: either enzymatic assays, or electrophoretic or immunological ones, but the three are never used together. Because of aldolase C activity, the enzymatic assays which measure this activity ratio Fru-1,6-P_2/Fru-1-*P* cannot give the proportions of aldolase A and B. There are considerable discrepancies in the various publications where these assays are used: Hommes and Draisma [17] found that the amount of M type, (which corresponds to the A type) is relatively constant, while the L type (or B type) increases with age. They found a great amount of aldolase M even in adult liver. Masters [11] found a very low activity ratio which does not change with the age of the animal. For this author, at the 12th day of the embryo, the activity ratio is 1.2, compared with 1.1 at birth and 1.1 in the adult. Walker and Van Potter

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[19] found a very high activity ratio (about 40) at the 17th day of fetal life and conclude that this fact is due to the hematopoïetic cells which would participate in the aldolase activity to a very great extent.

Simultaneously our data obtained by using the three types of methods, enzymatic, electrophoretic and immunological, gave us different results [20].

We found that at the 15th day of gestation, the aldolase activity ratio is close to 8.5 and goes down to 1.1 in the new born animal and to 1.0 in the 3-day-old (Table I).

TABLE I

ALDOLASE ACTIVITY IN FETAL LIVER AND NEWBORN RAT

Mean of 8 experiments.

Age of animals	Fru-1,6 P_2	Fru-1-P	Fru-1,6-P_2/Fru-1-P ratio
15th day of fetal life	8.3	0.99	8.25 ± 1.38
3-day-old	12.9	12.0	1.07 ± 0.04

Electrophoresis on cellulose acetate used by Susor and Rutter [22] and Rensing et al. [10] did not show the presence of aldolase C in fetal liver but only hydrid bands from aldolase A and B. Contrary to this we are able to detect the presence of aldolase C hydridized with aldolase A by electrophoresis on starch gel (Fig. 1). But both results are not sufficient evidence for the presence of aldolase C.

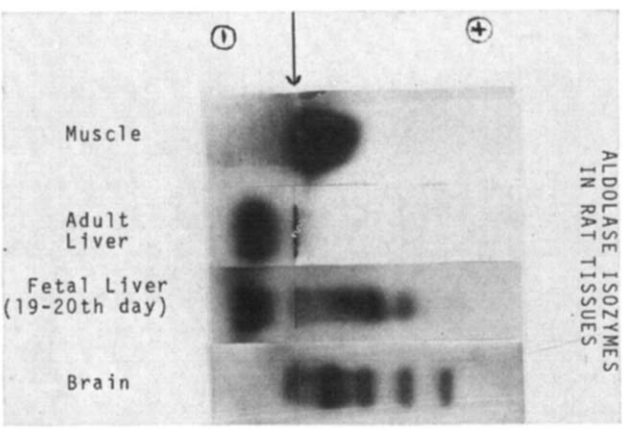

Fig. 1. Comparison of aldolase isozymes in rat tissues by starch gel electrophoresis in phosphate citrate buffer. Specific staining by reducing tetrazolium salts coupled to aldolase activity with the help of phosphoglyceraldehyde dehydrogenase, which used nicotinamide adenine dinucleotide as coenzyme.

The immunological methods are the most precise to detect the enzyme and to measure the percentage of each one, in spite of the existence of hybrids which react with antisera in almost the same way as pure tetramers do.

That is the way we can give evidence that in the rat fetal liver, at the 15th day

TABLE II

INHIBITION PERCENTAGE OF FRU-1,6-P_2 AND FRU-1-P ALDOLASES ACTIVITY IN FETAL LIVER BY ANTI-ALDOLASE ANTISERA

Age of animals	Anti A		Anti B		Anti C	
	Fru-1,6-P_2	Fru-1-P	Fru-1,6-P_2	Fru-1-P	Fru-1,6-P_2	Fru-1-P
15th day of fetal life	30–35	5	60–70	45–55	30–70	30–95
3-day-old	17–25	5	68–75	85–92	11–19	5–13

of gestation, the three aldolases A, B and C are present and not only aldolase A and B; then aldolase B increases at the expense of the aldolases A and C (Table II).

The study of organs having an hematopoïetic function like liver raised the following problem: are the enzymatic activities due in part to the hemapoïetic cells or to the hepatic cells only? A comparative electrophoresis with rat fetal liver at the 17th day of gestation and primary rat fetal liver cells culture at the third day of culture gives the same isozymic pattern, and it must be noted that these cultures are almost exclusively made up with hepatic cells.

Principally, in fetal liver the number of hematopoïetic cells remains constant from the 15th to the 18th day and then decreases afterwards as shown by Nagel [23] and Greengard [24]. On the 20th day the number of hematopoïetic cells is slightly greater than the number of hepatic cells, although the activity ratio Fru-1,6-P_2/

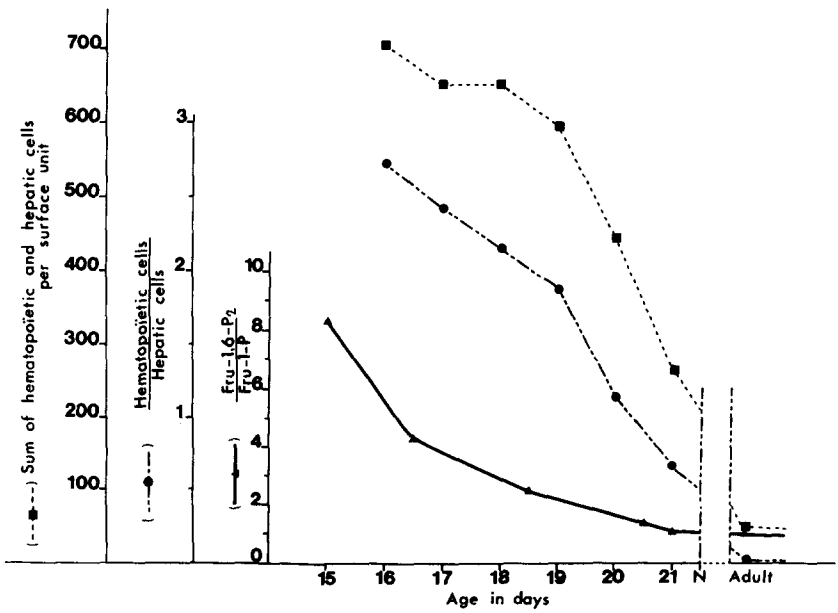

Fig. 2. Evolution of the hematopoïetic and hepatic cells as compared with the aldolase activity ratio. The aldolase activity ratio is measured according the colorimetric method of Sibley and Lehninger. The data about hepatic and hematopoïetic cells are taken from Nagel and Greengard et al. (See references).

Fru-1-*P* changes regularly from the 16th day onwards and is approximately equal to 1 at birth, that is to say it corresponds to a pure aldolase B without aldolases A and C (Fig. 2). So it does not seem probable that the high aldolase activity ratio found in fetal liver would be due to the hematopoïetic cells. Moreover, this ratio does not change when this hematopoïetic tissue regresses under the influence of a stress (unpublished results). These facts reflect more "the changing isozymic composition of parenchymal tissue and not the changing cellular composition of liver" [24]. The three types of aldolases A, B and C are present in rat fetal liver, while aldolase B only is present in adult: that is to say, aldolase B is characteristic of the hepatic cell differentiation. These three enzymes seem to be the product of three structural genes, which are translated together in vivo [25] and in vitro [26]. Evidence for two different structural genes for aldolase A and B is brought by Hereditary Fructose Intolerance [27].

In the adult, aldolase C is mostly localized in brain and its function is still not well known, although many structural (Lee, Y., Felicioli, R. and Horecker, B. L., personal communication) [28] and biochemical studies [28–30] were devoted to it. It is interesting to note that the enzymatic characteristics of this tissue do not change very much during the ontogenic evolution.

Perhaps aldolase C which is hybridized with aldolase A in the brain and in several fetal tissues has a specific physiological function for fetal life. But it may just be a residue from phylogenic evolution.

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