FRUCTOSE 1, 6 BISPHOSPHATASE ACTIVITIES IN MOUSE LIVER AS A FUNCTION OF AGE

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Summary : Neutral fructose 1, 6 bisphosphatase activity increases till 7 days, after which, a decline is observed postnatally upto 30 days. Alkaline fructose 1, 6 biphosphatase follows the same pattern.

The optimum activity of fructose 1, 6 bisphosphatase in mouse liver at pH 6.5 and 9.0 of all the periods, suggests the presence of both neutral and alkaline enzyme during the deve'opmental period studied. On the basis of similarity observed in optimum pH, the same properties of enzyme at all the developmental stages sudied, could not be ruled out.

Key words : age neutral alkaline fructose 1,6 bisphosphatase liver mouse

INTRODUCTION

The assumption of a parallel development of the enzyme activity and enzyme function has become a widely accepted fact. Several changes in hepatic enzymes have been recorded during development of several species (6). Taylor (15) observed that the activity of Fructose 1, 6 diphosphatase in rat liver reaches maximum at about 10 days but falls rapidly after weaning to reach adult value at about 30 days. The activities of fructose 1, 6 diphosphatase in pig liver increased four fold by second day, gradually rose to maximum value by day 14 and then declined (10). It has also been shown that the properties of fructose diphosphatase from embryonic livers were similar to the adult liver and the differences between the species were exceptionally small (16,17).

Present study has been carried out to observe the changes in the activity of fructose 1, 6 bisphosphatases (EC 3.1.3.11) in mouse liver, during development.

MATERIAL AND METHODS

Albino mice were procured from the colony raised in the departmental animal house by the inbreeding technique. Livers of mouse were analysed over the entire growth

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period following birth. Pooled liver tissues were collected from a number of animals of exactly same age group. A sufficient number of animals were used to have a minimum yield of 1.5 *gm* of tissue. A 20% homogenate was prepared in 0.25 M freshly prepared iso-osmotic sucrose solution with the help of Potter-Elvehjem homogenizer type 'A'. The homogenates were centrifuged at 15,000 g for 1 hour and the supernatant fractions were used as the source of enzyme. The assay systems for neutral and alkaline fructose bis-phosphatases and other details are the same as discussed earlier (12). Inorganic phosphate contents were determined colorimetrically (5). Protein content was determined by the method of Lowry *et al.* (8) using bovine serum albumin as standard.

RESULTS

The activities were calculated as specific activity (per mg protein) and as units per g liver. The expression of somatic tissue index (per liver from 100 gm body weight) has special significance from the physiological point of view, since the data reflect the contribution by this organ to the economy of the animal as a whole.

The data for body weight of the animal, absolute and relative weight of liver and protein values of homogenate and supernatant fractions are recorded in Table I.

TABLE I : Average weights of body, liver and the protein content of liver homogenate and supernatant (15,000 x g).

Days of growth	*Average weight of animal (gm)*	*Average weight of liver (gm)*	*Weight of liver per 100 gm body weight (gm) (Somatic tissue index)*	*Protein (homogenate mg/gm/tissue weight*	*Protein (Supernatant) mg/gm tissue weight*
1 (28)	1.6±0.06	0.05±0.001	3.1	139.0±9.7	61.2±4.3
4 (25)	2.8±0.12	0.09±0.001	3.2	130.0±12.2	55.0±4.5
7 (18)	3.9±0.19	0.10±0.002	2.7	128.5±9.3	55.5±5.0
15 (15)	7.7±0.68	0.28±0.009	3.7	132.0±10.7	60.5±4.6
30 (8)	10.5±0.89	0.45±0.022	4.2	140.0±14.7	66.8±6.0
90 (adult, 5)	32.6±3.10	1.49±0.096	4.5	159.0±12.6	74.1±6.4
240 (old, 3)	35.0±2.60	1.86±0.137	5.4	152.0±13.8	73.8±7.2

The number within parentheses represents the number of animals sacrificed for pooling of tissues.
The values expressed are the mean ±S.E. of four separate experiments.

A persistent weight gain as expected, was observed in the average animal body weight throughout the developmental period. However, the liver weight increased rapidly after one month of age. In general, there was no significant change in the protein values of weaning animals. A significant increase of 22 and 34% ($P<0.005$) was observed in the protein values of adult mice liver homogenate and supernatant (15,000 x g) fractions, respectively.

Analytical datas for fructose bisphosphatase activities are enumerated in Table II.

TABLE II : Neutral and alkaline fructose bisphosphatase activities in mouse liver supernatant (15,000 x g) in relation to age.

	Neutral fructose bisphosphatase activtiy		*Alkaline fructose bisphosphatase activity*	
Days of growth	*Units/gm liver*	*Specific activity (per mg protein)*	*Units/gm liver*	*Specific activity (per mg protein)*
1	31.1±1.7	0.506±0.021	35.3±2.9	0.571±0.032
4	44.0±3.1	0.800±0.056	45.5±4.1	0.827±0.059
7	65.6±4.4	1.182±0.101	62.6±4.8	1.127±0.099
15	50.0±4.0	0.826±0.065	46.2±2.7	0.760±0.054
30	36.8±2.3	0.550±0.043	42.1±3.6	0.628±0.032
90	30.5±2.7	0.412±0.024	38.7±3.0	0.522±0.040
240	26.6±1.8	0.260±0.025	35.5±2.6	0.581±0.022

The values expressed are the mean ±S.E. four separate determinations.

Neutral fructose 1, 6 bisphosphatase – The optimum enzyme levels were observed at the age of 7 days. Enzyme activities of one day old mice liver were the same as of adult. However, following birth a constant increase in the fructose bisphosphatase activity was observed up to 7th day of age which then declined progressively and returned to the level of one day old animal. The increase in enzyme levels on 7th day was about 1.8 fold per g liver and 2.3 fold per *mg* protein, respectively when compared with that of one day old animals.

Alkaline fructose bisphcsphatase – Similar to the neutral enzyme the activity of alkaline fructose bisphosphatase also increased upto 7th day following birth and then

TABLE III : Activity of fructose bisphosphatase at different pH in mouse livers of different age groups.

	Age					
pH	*1 day*	*4 days*	*7 days*	*15 days*	*30 days*	*90 days*
			Activity, Units/gm liver			
6.0	20.4±2.2	31.5±3.0	50.0±4.1	35.5±2.5	21.8±1.1	19.8±0.8
6.5	34.5±2.8	43.5±3.7	66.0±4.9	56.0±5.0	40.0±3.3	32.0±2.3
7.0	24.0±2.4	30.0±2.5	36.5±2.7	28.6±1.2	25.0±1.5	20.5±1.2
7.5	22.0±1.6	26.5±1.9	34.5±2.2	30.5±1.5	23.5±1.2	17.6±0.6
8.0	20.5±1.2	29.6±1.7	38.6±3.2	32.6±1.9	30.6±1.6	26.5±0.9
8.5	25.6±1.7	34.7±3.0	43.0±3.5	35.5±2.3	35.5±1.9	32.4±1.2
9.0	39.0±2.2	47.0±3.6	60.5±3.5	49.0±2.6	47.0±3.1	41.2±1.7
9.5	32.6±2.8	36.9±2.6	34.0±2.0	28.5±1.2	26.6±1.2	20.5±0.7

The values expresed are the mean ± S.E. of four separate determinations. Universal verenalacetate buffer (10 *μmoles*) was used for the determination of optimum pH.

declined to the level of one day old. animal. It also attained adult value on the very first day after birth. Maximum increase in activity (1.5 fold per g liver and 2.0 fold per mg protein) was observed in 7 days old mouse liver as compared to that of 24 hr old mouse liver.

DISCUSSION

It might be suggested that aging results from a failure in the regulation of transcription and translation of protein synthesis. Most of the studies on carbohydrate metabolism in pre and postnatal mammals have been centered on rat and sheep. The pattern of development of individual enzymes primarily concerned with gluconeogenesis is species specific. Developmental changes in enzyme activity may be due to either differences in enzyme protein content or changes in the specific activity of the enzyme molecules.

Neutral fructose 1, 6 bisphosphatase activity in mouse is found to increase till 7th day after which a decline is noted postnatally upto 30th day. Similar trend of enzyme activity has also been observed with alkaline fructose bisphosphatase. The activity of fructose diphosphatase has already been reported to increase during the suckling period

and decrease after weaning in contrast to lipogenesis (15). The activity of fructose diphosphatase, a key enzyme in gluconeogenesis, was found to be negligible in the foetal liver (1, 2, 15). The weaning period, in the present study, was taken as 30 days when the young ones were separated from the mother. However, it has been observed that the animals after 15 days of age do start nibbling the outside food supplied to the mother and by begnning of 5th week they are completely weaned (7). Thus, during this period the quantitative ratio of the main nutrients changes progressively, in particular the ratio of carbohydrate to fat with the intake of carbohydrate increasing substantially. During weaning period, there occurs a change of diet from high fat (47%), low protein (9.6%), low carbohydrate and milk to the diet of adults containing high protein and carbohydrate and low fat. A lower value for the activity of both neutral and alkaline fructose bisphosphatase is found in 30 days old animals as compared to those of 15 days of age. An increase of activity of this enzyme in liver when the supply of glucose is curtailed could be interpreted as a compensatory adaptation of increased gluconeogenesis in response to carbohydrate (glucose) feeding (14). Active gluconeogenesis seems to be more important than sparing of glucose by high concentration of fat derived substrates for the maintenance of blood glucose in sukkling new born rats (4).

It has been proposed that aging in animals is associated with a drop in the specific activity of key enzymes (18). A decrease is observed in both neutral and alkaline fructose bisphosphatase activity in the old animal. Sen and Gandhi (13) too observed that the activity of fructose diphosphatase is decreased in old rats. The presence of both neutral and alkaline fructose bisphosphatase is revealed in the present study during the development of mouse liver. The presence of fructose bisphosphatases with two pH optima (Table III) have also been reported earlier (3,9).

In the present study, the pH optima of 6.5 for neutral fructose bisphosphatase and pH 9.0 for alkaline fructose bisphosphatase was observed constantly throughout the developmental period. It indicates the similarity of the enzyme throughout the pre, post-weaning and adult period. Wallace and Newsholme (16) have also suggested a similarity between embryonic and adult liver fructose diphosphatases as regards the pH optima is concerned.

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