Effect of Homogenizing Medium and Nutritive State on Transaminase Activities of Tissues.* (24018)

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Determination of tissue enzyme activities by use of homogenates requires a medium which will free maximum enzyme activity in a soluble form. Some enzymes show higher activity when the tissue is homogenized in water (1-5) while others are more readily solubilized in isotonic solutions(6,7). A comparison has been made of the effectiveness of water and phosphate buffer in homogenization of tissues for aspartic- and alanine-glutamic transaminase activities in fed and postabsorptive rats and mice.

Methods. Animals: Mice were maintained in glass jars and rats in individual screen bottom metal cages in air-conditioned room at 22-24°C. They were fed *ad libitum* the Jim Dandy ration cubes. The mice were killed by dislocating the vertebrae at base of skull and the rats by blow on head. The animals were bled by cutting blood vessels of the neck. *Enzyme determinations*: Tissues were homogenized in an all glass apparatus at 0°C. Liver and heart were diluted to 1% and the kidney to 2%. Aspartic-glutamic transaminase activity was determined by the method of Cohen and Cammarata(8) with omission of pyridoxal phosphate and pre-incubations. The reactants were prepared in 0.05 M potassium phosphate buffer (pH 7.4) and preheated to 40° in water bath prior to use. Buffer was substituted for the α-keto-glutarate in the blank cell. Formation of oxalacetic acid was continuously measured for at least 5 minutes at 270 mμ with Cary Model 14 spectrophotometer† containing a thermostable cell-chamber maintained at 37°C. A comparison was made of the spectrophotometric method with the colorimetric method(9) and a factor derived to convert changes in optical density to units previously used(10). Alanine-glutamic transaminase activity was determined by modification(11) of a colorimetric method for aspartic-glutamic transaminase(9).

Results. Nutritive state: Removal of food on the day prior to autopsy caused a decrease in body and liver weights, a slight decrease in weight of kidneys and no effect on heart. Lack of food did not affect magnitude of transaminase activities in the kidney and heart (Tables I and II). Transaminase activities of liver changed in proportion with change in liver weight, except aspartic-glutamic transaminase in the mouse liver which maintained its total activity.

Buffer vs. water as homogenizing medium: Homogenization of tissues in buffer gave lower values for aspartic-glutamic transaminase activity in liver and kidney but higher values in the heart (Tables I, II). The alanine-glutamic transaminase activity on the other hand was the same in both media for heart and liver but that of kidneys was lower with the buffer preparation. The changes were practically identical for tissues of the rat and mouse in both fed and postabsorption states.

Discussion. The difference in response of the 2 transaminases to homogenization in water and buffer indicates a difference in solubility, tissue combination or denaturation of these enzymes. Furthermore, the degree and nature of the binding varies with the tissues. Both enzymes may be readily mobilized along with other proteins when the exogenous source of foodstuffs is withdrawn. Failure of mouse liver, however, to give up any of its aspartic-glutamic transaminase activity is noteworthy. Apparently, this enzyme is not spared as readily as the alanine-glutamic transaminase. Removal of food has been demonstrated to have variable effects on the activity of a number of enzymes(12,13).

Summary. An overnight fast resulted in decrease in weight of liver of rats and mice

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TABLE I. Effect of Homogenizing Medium and Nutritive State on Aspartic-Glutamic Transaminase Activities of Tissues.*

Homogenizing medium	Rat			Mouse		
	Liver	Kidney	Heart	Liver	Kidney	Heart
	u/g					
			Fed			
Water	143	97	200	236	118	298
Buffer†	128	73	263	152	88	350
Diff., %‡	-11	-25	+32	-35	-25	+17
			Fasted			
Water	156	92	196	316	119	296
Buffer†	128	83	263	205	92	360
Diff., %	-18	-10	+33	-35	-24	+21

* Male rats, Sprague-Dawley, 80 days old, 310 g body wt. Male mice, Holtzman, 220 days old, 44.6 g body wt. 5 animals/group. Significant changes are underlined. The same homogenates were used for both enzymes (Table II).

† .05 M potassium phosphate, pH 7.4.

‡ Mice (National Lab., 2 mo old) gave differences of -31, -29 and +31 for the respective tissues.

TABLE II. Effect of Homogenizing Medium and Nutritive State on Alanine-Glutamic Transaminase Activity of Tissues.*

Homogenizing medium	Rat			Mouse		
	Liver	Kidney	Heart	Liver	Kidney	Heart
	u/g					
			Fed			
Water	68	5.0	6.4	119	14.2	9.4
Buffer	68	4.4	6.3	121	9.9	8.8
Diff., %†	0	-11	-2	0	-30	-6
			Fasted			
Water	49	4.7	6.3	135	14.3	10.1
Buffer	59	3.5	6.2	122	11.3	8.8
Diff., %	(+20)	-25	-2	-9	-21	-13

* See Table I. Significant changes are underlined.

† Mice (Holtzman, 2 mo old) gave differences of: -6, -16, and 0% for the respective tissues.

with a proportionate decrease in their aspartic- and alanine-glutamic transaminase activities except for the aspartic-glutamic transaminase of mouse liver which maintained its total activity. Homogenates of liver and heart prepared in water had the same alanine-glutamic transaminase activities as those prepared in 0.05 M potassium phosphate (pH 7.4) but the kidney enzyme was lower in buffer. The aspartic-glutamic transaminase activities of liver and kidney were higher in water homogenates while that of the heart was higher in buffer.

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