tions that isopentenyladenosine inhibits growth of undifferentiated precursors of white blood cells in tissue culture (myeloblasts) (*18*), while it appears to have stimulated cell division in vivo when used in the therapy of acute myeloblastic leukemia in man (*9*). These findings might be explained by the results reported in this communication demonstrating that isopentenyladenosine can have both growth-promoting and growth-inhibiting effects on human cells, depending on the concentration and on the particular stage of the cell cycle. The results of these experiments do not warrant extensive speculations on the mechanism or mechanisms of isopentenyladenosine inhibition and stimulation. However, some observations suggest that the primary effect of isopentenyladenosine is on RNA synthesis.

Frequently, in the treatment of human neoplasias, antitumor agents directed against the dividing cell are not effective because of a low rate of DNA synthesis accompanied by a relatively low incidence of mitosis. It is possible that isopentenyladenosine might be useful in some instances not only for its direct antitumor effects but to help trigger some cells to divide. In addition, inhibition of PHA-induced lymphocyte blastogenesis by isopentenyladenosine suggests that isopentenyladenosine has potential immunosuppressive properties.

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Renal Fructose-Metabolizing Enzymes: Significance in Hereditary Fructose Intolerance

Abstract. *In patients with hereditary fructose intolerance, which is characterized by deficient aldolase activity toward fructose-1-phosphate, fructose induces a renal tubular dysfunction that implicates only the proximal convoluted tubule. Because normal metabolism of fructose by way of fructose-1-phosphate requires fructokinase, aldolase "B," and triokinase, the exclusively cortical location of these enzymes indicates that the medulla is not involved in the metabolic abnormality presumably causal of the renal dysfunction.*

Invariably and apparently uniquely in patients with hereditary fructose intolerance, the sustained administration of fructose induces, within 30 minutes, a complex of proximal tubular dysfunctions that occur simultaneously and persist throughout the administration of fructose (*1, 2*). Renal medullary function appears unaffected (*2, 3*). These findings accord with the hypothesis that fructose induces a metabolic abnormality in the renal cortex but not in the renal medulla (*2*).

In hereditary fructose intolerance, the metabolic abnormality induced by fructose is initiated by cellular accumulation of fructose-1-phosphate (F1P) in those tissues deficient in aldolase activity toward this substance: liver (*4, 5*), kidney (*6*), and small bowel (*7*). These organs normally extract fructose briskly and convert it to glucose, predominantly by way of F1P and the triose products of its aldolase cleavage (*8, 9*). Fructokinase (E.C. 2.7.1.3) catalyzes the phosphorylation of fructose to F1P and is intact in patients with hereditary fructose intolerance (*4*). The "B" isoenzyme of aldolase ("liver aldolase") (E.C. 4.1.2.7) has strong cleaving activity toward F1P and, in keeping with the glucogenic capacity of liver and kidney, strong condensing activity toward dihydroxyacetone phosphate and D-glyceraldehyde-phosphate (*10, 11*). Triokinase (E.C. 2.7.1.28) catalyzes the phosphorylation of D-glyceraldehyde, one of the two aldolase cleavage products of F1P (*12*). Since fructokinase and aldolase B appear to occur only in the liver, kidney, and small bowel (*10, 13*), only these organs could accumulate F1P because of deficient aldolase activity. That the renal medulla might lack these fructose-metabolizing enzymes is suggested by the observation that rabbit renal medulla extracts fructose sparingly (*9*) and converts none to glucose and by the observation that aldolase activity toward F1P in the renal medulla is a small fraction of that in the cortex (*2*).

Table 1. Fructokinase, triokinase, and aldolase activities in mammalian tissues. The activities of fructokinase and triokinase are expressed as the number of micromoles of adenosine triphosphate formed per gram of protein per minute. The activity of aldolase is expressed as the number of micromoles of fructose-1,6-diphosphate (FDP) or fructose-1-phosphate (F1P) utilized per minute per milligram of protein, times 100. All the activities are expressed as the means ± the standard error of the means; the number of specimens assayed is given in parentheses.

Tissue	Enzyme activities				FDP/F1P*
	Fructokinase	Triokinase	Aldolase		
			FDP	F1P	
		Dog			
Kidney : cortex	4.9 ± 0.7 (5)	9.7 ± 2.0 (5)	14.5 ± 2.8 (9)	8.4 ± 2.0 (9)	1.8
Kidney : medulla	0 (5)	0 (5)	4.3 ± 0.6 (9)	0.2 ± 0.02 (9)	20.6
Muscle	0 (2)	0 (2)	101.9 ± 9.9 (5)	4.1 ± 0.5 (5)	25.3
Liver	2.7 ± 0.4 (2)	6.5 ± 0.9 (2)	8.5 ± 2.6 (5)	6.6 ± 2.1 (5)	1.4
		Human			
Kidney : cortex	4.7 (1)	10.0 (1)	3.2 (1)	2.5 (1)	1.3
Kidney : medulla	0 (1)	0 (1)	7.7 (1)	0.3 (1)	22.6

* Ratio of aldolase activity toward FDP and F1P.

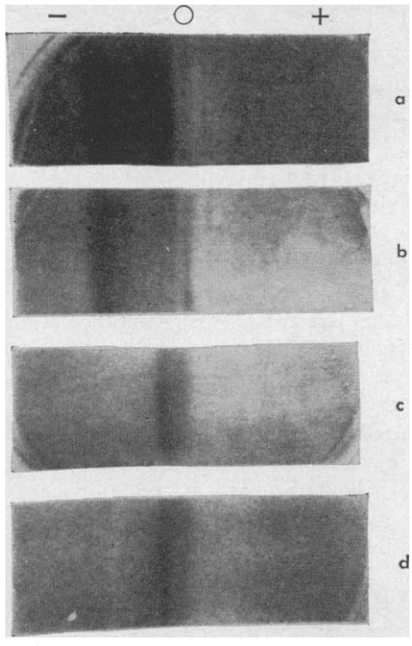

Fig. 1. Patterns of aldolase activity of adult goat tissues after zone electrophoresis. (a) Liver, (b) renal cortex, (c) renal medulla, and (d) muscle.

We now report that only aldolase A (E.C. 4.1.2.13) ("muscle aldolase") could be demonstrated in normal mammalian renal medulla, whereas the renal cortex contained the predicted cluster: fructokinase, aldolase B, and triokinase.

Specimens of liver, kidney, and muscle were obtained from various mammals. From whole kidneys, the cortex was separated from the inner medulla. The tissues were minced and homogenized in equal volumes of 0.25*M* mannitol or 0.06*M* barbital buffer at *p*H 8.6, and the resulting homogenates were centrifuged at 100,000*g* for 60 minutes. The clear supernatant was used for the determination of the activities of fructokinase, aldolase, and triokinase and for determining the electrophoretic mobilities of aldolase. Aldolase activity was assayed by the methods of Rutter and associates with fructose-1,6-diphosphate (FDP) and F1P as substrates (*14*). Fructokinase and triokinase were assayed by the methods of Adelman and co-workers (*13, 15*). A micromethod for the determination of protein was adapted from Oyama and Eagle (*16*). Zone electrophoresis was performed in 0.06*M* barbital, 0.01*M* β-mercaptoethanol, *p*H 8.6, on cellulose acetate strips at 250 volts for 120 minutes with the origin equidistant from the electrodes. The strips were stained for aldolase activity as described (*17*).

In the tissues examined, the specific activity of aldolase toward F1P was highest in the renal cortex and lowest in the renal medulla, the difference being greater than an order of magnitude (Table 1). The FDP-F1P activity ratios in the renal cortex were 1.3 and 1.8 in the human and the dog, respectively, and are similar to those in liver, where more than 98 percent of the aldolase protein is aldolase B (*17*). The FDP-F1P activity ratios of 20.6 and 22.6 in the renal medulla are similar to the activity ratio of 25 in muscle, which contains only aldolase A (*18*). Similar results were obtained in five goats and one pig. Zone electrophoresis of extract from renal cortex demonstrates the presence of both A and B as does that from the liver, whereas renal medullary and muscle aldolase have apparently identical mobilities (Fig. 1). In this system relatively low concentrations of aldolase A can be detected (*19*).

This would explain why in liver, where more than 98 percent of aldolase is aldolase B, both A and B are readily discernible. These data indicate that renal cortical aldolase is predominantly B while renal medullary aldolase is A. Fructokinase and triokinase activities were demonstrated in the renal cortex and liver, but not in renal medulla and somatic muscle.

The finding of fructokinase, aldolase B, and triokinase in the renal cortex but not in the medulla constitutes strong evidence that normally only the cortex metabolizes fructose by way of FlP and its aldolase cleavage. Accordingly, deficient renal aldolase cleavage of FlP would impair only renal cortical metabolism of fructose (*20*) and thereby explain why only cortical function might be disrupted in patients with hereditary fructose intolerance given fructose. The occurrence of medullary nephrocalcinosis and classic renal tubular acidosis in a patient with hereditary fructose intolerance (*21*) suggests that a disorder of the renal medulla may stem from an enzyme defect in the renal cortex.

That the renal cortex, like liver, contains aldolase B is consistent with the hypothesis that the enzymatic lesion in hereditary fructose intolerance is a defect in aldolase B protein (*22*) or an absence of aldolase B with fetal aldolase (aldolase A) persisting instead (*23*). But, the possibility cannot be excluded that, in some patients with hereditary fructose intolerance, all aldolase protein cannot cleave F1P normally. The absence of cleaving activity would be metabolically significant only in tissue which normally contains aldolase B, because only these tissues also contain fructokinase.

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19. In this activity stain aldolase is coupled with triosephosphate dehydrogenase (E.C. 1.2.1.12) so that cleavage of FDP results in the formation of reduced nicotinamide adenine dinucleotide (NADH) which ultimately leads to the reduction of nitroblue tetrazolium. Aldolase A is more readily stained than aldolase B because the maximum velocity (V_{max}) of aldolase A toward FDP is greater than ten times that of aldolase B.
20. In the studies demonstrating modest extraction of fructose by the rabbit renal medulla (*11*), 10 m*M* fructose was incubated with rabbit medullary slices in the absence of glucose. Since the Michaelis constant (K_m) of hexokinase for glucose is $10^{-5}M$ and for fructose is $10^{-3}M$, it seems probable that, at physiologic concentrations of glucose, the renal medulla extracts little, if any, fructose and that the small amount of fructose extracted was converted to fructose-6-phosphate.
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