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Comparative Metabolic Responses of Rat Kidney and Liver to Acute Doses of Fluoride[1] (38071)

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Little is known in man or experimental animals about the biochemical mechanism of acute inorganic fluoride toxicity. In man the symptoms of fluoride toxicity may occur during accidental or occupational overexposure to dietary or airborne fluorides (6). In experimental animals the physiological effects (i.e., depressed food intake, growth retardation, mottled enamel, skeletal changes, mortality) of toxic amounts of inorganic fluoride have been studied extensively (6, 7, 13), and many enzymes have been shown to be inhibited *in vitro* by fluoride (2, 14). However, the specific enzymatic or metabolic changes responsible for either the chronic or the acute toxicity of inorganic fluoride in the intact animal remain obscure despite recent extensive studies on liver carbohydrate (8–10), lipid (16), and amino acid (11) metabolism.

It has been pointed out that the kidney is quite sensitive in its histopathological and functional responses to toxic amounts of fluoride (6), but there have been very few studies on possible metabolic changes occurring in the kidneys from intact animals receiving elevated amounts of fluoride. Thus, the purpose of the study described below was to compare the effects of fluoride on the glycolytic and citric acid cycle pathways in the livers and kidneys from rats receiving acute doses of sodium fluoride.

Materials and Methods. Female Holtzman rats (Madison, Wisconsin) weighing between 175–200 g were individually caged in raised, screen bottom cages in a temperature (22°C) and light controlled (12 hr light—12 hr dark) animal room. Nonfluoridated tap water and a low fluoride, semisynthetic casein-sucrose diet (12) were available *ad libitum* several days before the administration of fluoride. Between 9:00 a.m. and noon of the experimental days the rats received a single intraperitoneal injection of approximately 0.5 ml of one of the following solutions: control rats, saline; nonlethal fluoride treated rats, 10 mg F (as NaF)/kg body weight; and lethal fluoride treated rats, 25 mg F/kg. Preliminary experiments showed that 10 mg F/kg was not lethal and that 25 mg F/kg was lethal within 1 hr. At either 15 min or 90 min after each injection, the rats were killed by cervical fracture, and the liver or both kidneys were rapidly removed in less than 10 sec and frozen in liquid nitrogen.

In order to study the effect of fluoride on the glycolytic and citric acid cycle pathways, 9 glycolytic intermediates, 3 citric acid cycle intermediates, and ATP were measured in neutralized perchloric acid extracts of the kidneys and livers by enzymatic methods previously described (1, 9). Fluoride ion concentrations in neutralized perchloric acid extracts of the livers and kidneys were measured with the Orion combination fluoride specific ion electrode (model number 96-09) by the single standard addition method (5, 9). The validity of this method for our sample matrix was established as suggested by Hall *et al.* (5) by confirming the theoretical Nerst slope response of the electrode in kidney extract.

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TABLE I. Liver Metabolites and Fluoride Concentrations in Rats 15 Min After a Lethal Injection of Fluoride.

	Control	Fluoride treated
Metabolites	μmoles/g	
Glucose	4.774 ± 0.194 (6) [a]	4.434 ± 0.199 (5)
G-1-P	0.003 ± 0.002 (7)	0.002 ± 0.001 (7)
G-6-P	0.290 ± 0.018 (7)	0.350 ± 0.009 (7) [b]
F-6-P	0.057 ± 0.004 (7)	0.074 ± 0.006 (7) [b]
3-PGA	0.286 ± 0.056 (7)	0.688 ± 0.067 (7) [d]
2-PGA	0.025 ± 0.010 (7)	0.088 ± 0.021 (7) [b]
PEP	0.057 ± 0.001 (7)	0.174 ± 0.032 (7) [c]
Pyruvate	0.128 ± 0.017 (7)	0.064 ± 0.016 (7) [b]
Lactate	1.455 ± 0.172 (7)	0.648 ± 0.188 (7) [c]
Citrate	0.333 ± 0.026 (7)	0.185 ± 0.015 (7) [d]
α-KG	0.077 ± 0.009 (5)	0.027 ± 0.002 (5) [d]
Malate	0.339 ± 0.042 (5)	0.280 ± 0.022 (5)
ATP	1.645 ± 0.193 (5)	1.951 ± 0.128 (5)
	ppm	
Fluoride	0.27 ± 0.03 (5)	26.69 ± 1.20 (5)

[a] Mean ± standard error. Number of observations in parentheses.

[b] The difference between the means of the control and fluoride groups were significant at $P < 0.05$.

[c] $P < 0.01$.

[d] $P < 0.001$.

Results. The metabolic responses of the liver and kidneys from rats receiving lethal amounts of fluoride, 25 mg/kg, were remarkably different. In the liver (Table I), fluoride caused statistically significant changes in the concentrations of these 9 metabolites[2] (expressed as percent change): G-6-P, +21%; F-6-P, +30%; 3-PGA, +141%; 2-PGA, +252%; PEP, +205%; pyruvate, −50%; lactate, −56%; citrate, −45%; and α-KG, −65%. Although there were large changes within the liver glycolytic and citric acid cycle pathways, glucose and ATP concentrations were not altered by fluoride. Liver fluoride concentrations in the fluoride treated rats were increased to 27 ppm compared to 0.27 ppm F for the controls.

In the kidneys of rats receiving 25 mg F/kg (Table II), glucose concentrations were increased, but in contrast to the marked metabolic response in the liver, fluoride caused no statistically significant change in the concentration of any other kidney glycolytic or citric acid cycle intermediate. Apparent kidney fluoride concentrations, 52 ppm, in the fluoride injected rats were approximately double the liver fluoride concentrations.

The fluoride animals were obviously morbund 15 min after receiving 25 mg F/kg, and by 60 min they would have expired. Therefore, a nonlethal dose of fluoride, 10 mg/kg, was also injected into rats so that the effect of fluoride on kidney metabolism could be tested after a longer time period, 90 min. The results were similar to those obtained using fatal amounts of fluoride; no statistically significant changes were found in any of the glycolytic intermediates or in citrate concentrations. Apparent kidney fluoride levels were 4.11 ± 1.01(5) ppm in the fluoride treated animals.

[2] Abbreviations used are: G-1-P, glucose-1-phosphate; G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; 3-PGA, 3-phosphoglycerate; 2-PGA, 2-phosphoglycerate; PEP, phosphoenolpyruvate; α-KG, α-ketoglutarate; ATP, adenosine-5′-triphosphoric acid.

TABLE II. Kidney Metabolites and Fluoride Concentrations in Rats 15 Min After a Lethal Injection of Fluoride.

	Control	Fluoride treated
Metabolites	μmoles/g	
Glucose	3.532 ± 0.056 (5) [a]	8.164 ± 0.191 (2) [b]
G-1-P	0.006 ± 0.003 (6)	0.002 ± 0.001 (6)
G-6-P	0.028 ± 0.009 (6)	0.034 ± 0.014 (6)
F-6-P	0.013 ± 0.006 (6)	0.031 ± 0.013 (6)
3-PGA	0.259 ± 0.038 (6)	0.403 ± 0.062 (6)
2-PGA	0.114 ± 0.032 (6)	0.151 ± 0.044 (6)
PEP	0.143 ± 0.026 (6)	0.201 ± 0.061 (6)
Pyruvate	0.137 ± 0.031 (6)	0.130 ± 0.040 (6)
Lactate	1.062 ± 0.101 (6)	1.146 ± 0.174 (6)
Citrate	0.535 ± 0.038 (6)	0.502 ± 0.016 (6)
α-KG	0.195 ± 0.028 (6)	0.139 ± 0.017 (6)
Malate	0.312 ± 0.036 (6)	0.307 ± 0.021 (6)
ATP	0.966 ± 0.115 (6)	0.961 ± 0.160 (6)
	ppm	
Fluoride	0.83 ± 0.44 (5)	51.53 ± 1.92 (6)

[a] Mean ± standard error. Number of observations in parentheses.

[b] The difference between the means of the control and fluoride groups were significant at $P < 0.001$.

Discussion. The lack of effect of both lethal and nonlethal amounts of fluoride on kidney metabolism noted in the present experiment was quite unexpected. Numerous structural and functional changes, reviewed elsewhere (6), have been noted in the kidneys of animals receiving increased amounts of fluoride under a variety of conditions. The studies reported here are, however, the first systematic search along two biochemical pathways, glycolysis and citric acid cycle, of kidney for metabolic lesions caused by acute amounts of fluoride. It has been pointed out that functional kidney injury is not the cause of death in acute fluoride poisoning (6) and that kidney injury is of minor importance in the fluorosis syndrome (15). The metabolic resistance of the kidney to fluoride noted in the present experiment provides a biochemical basis for these observations.

Furthermore, the present metabolic studies were performed on liver and kidney tissue containing some of the highest soft tissue fluoride concentrations reported in the literature (6). Lethal plasma fluoride concentrations were recently reported to be 28 ppm F in rabbits and 10–12 ppm F in rats (4). However, fluoride is rapidly filtered into the urine by the kidney (6), and the high kidney fluoride levels found in the present experiment were undoubtedly due to the fact that some of the fluoride was present as urine. The excretion of fluoride may protect intracellular intermediary metabolism in the kidney against the detrimental effects of fluoride such as observed in the liver.

The marked changes in liver glycolytic and citric acid cycle intermediates produced by lethal amounts of fluoride in the present experiment indicated enzyme inhibition by fluoride. It was recently reported that the *in vitro* inhibition of enolase enzyme (EC 4.2.1.11), which catalyzes the conversion of 2-PGA to PEP, can occur as low as 0.5 ppm F (3). In light of the high liver fluoride concentrations found in the present experiment, it is likely that enolase was also inhibited in the livers of the fluoride treated rats. The build up of liver metabolites behind PEP and the fall in metabolites after pyruvate also noted in the present experiment has been shown to be due to an in-

direct effect of fluoride on pyruvate kinase enzyme (EC 2.7.1.1) (9).

Summary. The metabolic responses of kidney and liver to acute doses of sodium fluoride were compared in the intact rat. In the liver, fluoride caused numerous changes in the concentrations of glycolytic and citric acid cycle intermediates indicating enzyme inhibition by fluoride. In the kidneys, fluoride caused no statistically significant changes in the concentrations of metabolic intermediates, except glucose. It is concluded that in the intact animal the kidney is more metabolically resistant to acute doses of fluoride than the liver.

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