Action of Fluoroacetate on Uptake of PAH by Renal Slices of the Dog.* (20597)

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In a previous publication(1) we have shown that treatment of the anesthetized dog with sodium fluoroacetate inhibited renal p-aminohippurate (PAH) secretion as well as glomerular filtration rate and blood flow through the kidney(1). These hemodynamic changes made interpretation rather difficult and it was deemed desirable to determine the effects of fluoroacetate on PAH secretion by the method of Cross and Taggert(2). Cross and Taggert(2) have shown that the slice:medium ratio of PAH is an index of the secretory activity of the renal slices. In most of our experiments both the slice:medium ratio (S:M ratio) for PAH as well as the oxygen consumption have been determined in the presence and absence of acetate as substrate.

Methods. Dogs weighing 6 to 14 kg were anesthetized with 30 to 35 mg per kg of pentobarbital given intravenously. The left kidney was removed first and the S:M ratio and oxygen consumption were determined. The animal then received an infusion of isotonic saline, 3.4% sodium bicarbonate or 2 or 6% sodium acetate containing pentobarbital (50 mg/l.). The rate of infusion was about 10 cc /minute/m^2 body surface. About 30 minutes after the infusion was started either saline or fluoroacetate was injected intravenously. About 3 hours after this injection the right kidney was removed and the oxygen consumption and PAH slice:medium ratio were determined. The same technic as described by Cross and Taggert(2) was used except that the PAH concentration was 0.0013M. In some of the experiments the slices were incubated in a Dubnoff metabolic shaking machine and the gas phase used was 100% oxygen.

Results. Control experiments have shown that the oxygen consumption and the slice:medium ratio of PAH of the left and right kidney were about the same. The procedure used was the same as for the fluoroacetate experiments except that saline was given instead of fluoroacetate (Table I). From Table II it can be seen that the higher dosages of fluoroacetate depressed both oxygen consumption and PAH uptake in the absence of substrate and completely eliminated the increment in PAH uptake and oxygen consumption produced by acetate. With doses of 0.5 and 0.25 mg of NaFAc per kg the influence on oxygen consumption and PAH uptake in the absence of substrate was minimal but the increment produced by acetate as substrate, was practically completely eliminated. Table I shows the results when acetate was infused. Acetate infusions alone increased both oxygen consumption and the S:M ratio. The infusion of acetate protected the kidney against fluoroacetate and this protection was roughly proportional to the rate of infusion of sodium acetate (Table III). Control experiments with bicarbonate infusions did not show any protection against NaFAc and are included in Table III.

Discussion. It is apparent from the above findings that the metabolic reactions involved in the increment in oxygen consumption and S:M ratio produced by acetate are far more sensitive to fluoroacetate poisoning than the metabolic reactions occurring in the absence of acetate. It must be concluded that fluoroacetate primarily blocks those metabolic pathways related to acetate oxidation but is less effective in blocking the endogenous metabolism. Fluoroacetate resistant energy producing mechanisms have been demonstrated in intestinal(3-5) and in uterine(6) smooth muscle. It is possible that the kidney may have such a fluoroacetate resistant pathway thus explaining the relative resistance of the endogenous oxygen consumption and PAH uptake to NaFAc in the absence of added ace-

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TABLE I. Influence of Saline and Acetate Infusions on Oxygen Consumption and PAH Uptake of Dog Kidney Slices. Saline and acetate infusions about 10 cc/min./m² body surface. Left kidney (control) removed first. Three hours after start of infusion right kidney removed.

	Left kidney (control)				Right kidney (experimental)			
	Oxygen consumption		PAH S:M ratio		Oxygen consumption		PAH S:M ratio	
Infusion, %	No substrate added	NaAc, .01 M	No substrate added	NaAc, .01 M	No substrate added	NaAc, .01 M	No substrate added	NaAc, .01 M
.9 NaCl	.984	1.531	4.4	12.7	.960	1.576	4.2	11.8
" "	.781	1.370	3.6	9.5	.792	1.394	3.7	9.0
2 NaAc	—	—	3.59	8.76	—	—	7.2	8.0
" "	.683	1.130	3.3	7.4	.931	1.24	6.4	8.3
6 NaAc	.912	1.473	3.2	9.6	1.420	1.400	9.4	9.2
" "	1.02	1.625	4.0	11.5	1.715	1.532	10.8	12.0
" "	—	—	4.3	10.5	—	—	12.3	11.5
" "	—	—	4.2	10.4	—	—	9.11	9.4

TABLE II. Influence of Sodium Fluoroacetate on Oxygen Consumption and PAH Uptake of Dog Kidney Slices. Saline infusion about 10 cc/min./m² body surface. Left kidney removed first as control. Three hours after injection of saline or fluoroacetate right kidney removed.

	Left kidney (control)				Right kidney (experimental)			
	Oxygen consumption		PAH S:M ratio		Oxygen consumption		PAH S:M ratio	
Dosage of NaFAc, mg/kg	No substrate added	NaAc, .01 M	No substrate added	NaAc, .01 M	No substrate added	NaAc, .01 M	No substrate added	NaAc, .01 M
0	.984	1.531	4.4	12.7	.960	1.576	4.2	11.8
0	.781	1.370	3.6	9.5	.792	1.394	3.7	9.0
.25	1.04	1.632	6.8	17.2	1.00	1.18	6.45	6.8
.25	.845	1.276	4.0	9.7	.800	.901	4.2	6.05
.25	.712	1.140	3.7	8.6	.760	.73	3.5	4.0
.5	1.19	1.570	7.0	16.07	1.00	.925	3.10	2.45
.5	.69	1.21	4.0	11.70	.71	.820	3.14	4.54
.75	.915	1.31	2.6	5.43	.785	.790	1.4	1.59
1	.884	1.28	3.5	9.86	.715	.731	2.3	2.01
1	.904	1.465	3.9	10.70	.76	.742	1.85	2.05
2	.773	1.211	4.4	13.5	.525	.510	1.93	1.6

TABLE III. Influence of Sodium Acetate Infusions on Inhibition of Oxygen Consumption and PAH Uptake Produced by Sodium Fluoroacetate. Left kidney was removed first and used as control. Infusion of sodium acetate or bicarbonate was started (about 10 cc/min./m²) and 30 min. later 1 mg sodium fluoroacetate/kg given. Three hours after fluoroacetate administration right kidney removed and studied.

	Left kidney (control)				Right kidney (experimental)			
	Oxygen consumption		S:M ratio		Oxygen consumption		S:M ratio	
Infusion, %	No substrate needed	NaAc, .01 M	No substrate needed	NaAc, .01 M	No substrate needed	NaAc, .01 M	No substrate needed	NaAc, .01 M
NaAc 2	.795	1.331	4.1	11.5	1.390	1.415	6.4	8.2
2	.937	1.674	3.5	8.2	1.610	1.572	4.8	6.5
6	.882	1.248	3.2	9.6	1.331	1.295	10.5	9.3
6	.768	1.310	2.9	9.0	1.285	1.335	10.3	12.0
6	.912	1.440	4.4	12.6	1.518	1.490	11.7	8.8
$NaHCO_3$ 3.4	—	—	7.9	14.6	—	—	2.24	2.20
" "	—	—	4.0	11.8	—	—	1.7	2.0

tate. Attempts are now being made to settle this point by adding fluoroacetate to kidney slices *in vitro*.

The infusion of acetate in the intact dog resulted in an increase in the oxygen consumption and PAH accumulation of the kid-

ney slices obtained from these dogs. This indicates that enough acetate or 2 carbon fragments had accumulated during the infusion period to stimulate the uptake of PAH to the same extent as 0.01M acetate added *in vitro* (compare Table I and III). The infusion of acetate further protected the kidney from NaFAc inhibition and the protection was roughly proportioned to the rate of acetate infusion.

The effect of fluoroacetate on oxygen consumption was relatively small compared to its effects on PAH accumulation. This is especially apparent in those experiments where no acetate was added. This indicates that only a small fraction of the total oxygen consumption is utilized for PAH transport. On the other hand fluoroacetate eliminated both the acetate induced increments in oxygen consumption and PAH uptake. This finding shows that acetate or the two carbon fragment is of primary importance in renal PAH transport(2).

Summary. Small doses of fluoroacetate mainly inhibit the ability of dog renal slices to respond to acetate stimulation as measured by PAH uptake and oxygen consumption. Larger doses of fluoroacetate produced inhibition of oxygen consumption and PAH uptake of renal slices in the absence of substrate. The effects on PAH uptake are more marked than are those on oxygen consumption.

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Stability of Serum Amylase.* (20598)

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Data available on the stability of pancreatic amylase in aqueous solution(1,2) indicate that highly purified preparations are quite stable but that crude preparations may lose their activity rapidly. However, a search of the literature disclosed a paucity of information regarding the stability of amylase in body tissues or fluids during storage. Scharles and Salter(3) state that aqueous extracts of the tumors of mouse sarcoma 180, preserved with toluene and kept in the refrigerator, retained their activity for several months. Fennel(4) states "urine or serum properly obtained and stored in the icebox at 4°C is trustworthy for 24, 48, or even 72 hours . . . the amylase content of serum remains remarkably constant in storage at 4°C; that of urine is not so stable." However, no quantitative data to support these statements were given in these papers and none was found elsewhere.

In clinical laboratories, it may be necessary to delay serum amylase determinations several days. Therefore, the question arises whether the amylase activity of stored sera remains constant.

Procedure. Samples of human blood were obtained from 14 normal, presumably healthy, staff members, technicians and secretaries of the University of Louisville School of Medicine. After clotting of the blood, the sera were immediately separated by centrifugation and analyzed for amylase activity by the method of Van Loon(5). Determinations of amylase activity were carried out immediately after separation of the sera (Day 1), one day

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