THE ACTION OF SODIUM FLUOROACETATE ON THE RENAL TUBULAR TRANSPORT OF PARA-AMINOHIPPURATE AND GLUCOSE IN THE DOG

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Fluoroacetate interferes with the oxidation of acetate and fatty acids and this block manifests itself by the accumulation of citrate in the poisoned tissues (Liebecq and Peters, 1948; Martius, 1949; Buffa and Peters, 1949; Potter and Bush, 1950; Kandel, Johnson and Chenoweth, 1951). The cause of citrate accumulation is probably the blocking of aconitase (Lotspeich, Peters and Wilson, 1952) by some fluorotricarboxylic acid formed from fluoroacetate (Buffa, Peters and Wakelin, 1951). Other sites of action of high concentrations of NaFAc have been described by Bush and Potter (1952).

Chenoweth (1950) in his review on fluoroacetate does not mention any studies on the kidney with fluoroacetate except the finding of Himwich (1950) that glucose Tm is not affected by a lethal dose of sodium fluoroacetate (NaFAc). Cross and Taggart (1950) have shown that the *in vitro* uptake of para-aminohippurate (PAH) by rabbit kidney slices is depressed by fluoroacetate. Because of the central importance of acetate in the secretion of PAH (Mudge and Taggart, 1950; Cross and Taggart, 1950; Shideman and Rene, 1951) and because of the relatively high specificity of sodium fluoroacetate inhibition it was of interest to study its effect on the renal transport of PAH and glucose in the dog. A preliminary report on this subject was published (Graham and Farah, 1952).

METHODS. In unanesthetized animals fluoroacetate produced severe convulsions in amounts less than 0.1 mgm. per kgm. In preliminary experiments it could be shown that dogs anesthetized with pentobarbital withstood larger doses of fluoroacetate and with these dosages effects on the kidney could be demonstrated. In the present experiments dogs were anesthetized with sodium pentobarbital (30 mgm. per kgm. intravenously followed by a continuous infusion of 0.05 mgm. per kgm. per minute). Para-aminohippurate, creatinine or inulin and glucose were added in adequate amounts to the solution which was infused at a rate of about 10 ml. per minute per m^2 of body surface. Plasma para-aminohippurate concentrations used were 38 to 109 mgm. per cent. PAH Tm or PAH secreted and glucose Tm were calculated by conventional methods, and 0.92 was considered to be the diffusible fraction of plasma PAH (Taggart, 1951). After three to four half hour control periods freshly dissolved fluoroacetate[2] was injected intravenously. Following the injection of sodium fluoroacetate usually four to eight half or one hour clearance periods were determined. In some of the experiments sodium acetate or bicarbonate solutions containing para-aminohippurate, creatinine and glucose were infused into the animal. In another series of

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experiments sodium acetate or monoacetin (glycerol monoacetate) were infused intravenously or injected intramuscularly two to three hours after the administration of NaFAc.

Para-aminohippurate was determined by the method of Smith *et al.* (1945), creatinine by the method of Folin and Wu (1919), inulin by the method of Schreiner (1950) and glucose by the method of Nelson (1944).

Renal blood flow was determined in three anesthetized dogs weighing 8.2–17.0 kgm. A bubble flow meter (Dumke and Schmidt, 1943) was attached to a common carotid and the left renal artery and arterial pressure was recorded by means of a mercury manometer. Heparin, in a dose of 3–5 mgm. per kgm. was used as an anticoagulant and following a number of control readings 2 mgm. of fluoroacetate per kgm. were given intravenously.

The renal load of PAH was determined by multiplying the plasma PAH concentration by the renal plasma flow. The latter value was not determined directly and thus we have multiplied glomerular filtration by 4 since 25 per cent was considered to be the filtration fraction. This manner of calculating PAH load is admittedly only a rough approximation and only marked deviations from the control curves would be of any significance.

In a number of dogs anesthetized with pentobarbital the left kidney was removed and the *in vitro* PAH uptake of kidney slices was measured by the slightly modified method of Cross and Taggart (1950). NaFAc was then given intravenously and three hours later the second kidney was removed and the PAH uptake of the kidney slices was determined by the same method.

The buffer used was the same as that of Cross and Taggart (1950); however, the PAH concentration was 0.0013 *M*. The slices were incubated in a Dubnoff metabolic shaking machine at a temperature of 25°C. Slices weighing 100 to 150 mgm. were incubated in a buffer containing no substrate while another set of slices was incubated in a similar buffer containing sodium acetate (0.01 *M*) as substrate. Two hours following a 15-minute equilibration period the slices were removed, weighed and placed in a trichloracetic acid solution. The PAH concentration in the slices and the medium was determined by the same methods as those described by Cross and Taggart (1950). The slice medium ratio was calculated on the basis of the final wet weight of the kidney slices. Recovery of PAH in these experiments was practically complete (99.0 ± 2.5 per cent).

RESULTS. Renal plasma flow (RPF) was determined by means of PAH clearance at low plasma concentrations and sodium fluoroacetate in a dose of 0.5 to 2.0 mgm. per kgm. produced a marked reduction in PAH clearance. This could be due to an actual reduction in renal plasma flow, back diffusion of PAH, or a reduction in the percentage extraction of PAH from plasma. The latter factor may be due to a disturbance in renal transport produced by fluoroacetate. Extraction of PAH from renal blood was not determined in these experiments. The fact that creatinine and inulin clearances were also reduced by fluoroacetate made it likely that the reduction in PAH clearance was at least partially due to an actual reduction in renal plasma flow. It was thus deemed desirable to determine renal blood flow by a direct method. We have used a modified bubble-flow meter after Dumke and Schmidt (1946). In all, three such experiments were conducted and 2 mgm. per kgm. of sodium fluoroacetate resulted in a reduction in renal blood flow which amounted to about 30–60 per cent of the control values (table 1). In all three experiments, fluoroacetate resulted also in some reduction in blood pressure (table 1) and it is possible that the reduction in renal blood flow was caused by this reduction in the perfusion pressure.

Glomerular filtration was determined by creatinine or inulin clearance. In both instances NaFAc resulted in a reduction in filtration rate which was quite varia-

ble but was related to the dosage of NaFAc (table 2). In a number of experiments the reduction in creatinine clearance was less than 20 per cent (fig. 1B) although a large dose of fluoroacetate had been injected.

PAH Tm. The action of fluoroacetate on PAH Tm was studied in fourteen dogs receiving isotonic NaCl infusions using doses of 0.25 to 2.0 mgm. of NaFAc per kgm. of body weight. With 0.25 mgm. per kgm. the effect on PAH Tm was relatively insignificant. With doses of NaFAc above 0.5 mgm. a marked reduction in PAH Tm could be produced. Concomitant with the reduction in PAH Tm there usually was also a reduction in creatinine or inulin clearance. However, in most instances the change in PAH Tm was greater than the reduction in glomerular filtration (fig. 1A, table 2). In two experiments, the reduction in

TABLE 1

The effect of NaFAc on renal blood flow in the dog

Female dog, 8.2 kgm. Pentobarbital anesthesia. Heparinized. Blood flow determined in the left renal artery by means of a bubble flow meter.

TIME IN MINUTES	BLOOD FLOW, ML. PER MIN.	BLOOD PRESSURE, MM. Hg	REMARKS
0	128	122	
5	108	120	
8	116	124	Heparin, 5 units I. V.
16	122	120	
22	120	120	
23	—	120	NaFAc, 2 mgm./kgm.
30	118	120	
40	110	120	Heparin, 5 units I. V.
48	96	118	
60	104	118	
73	80	114	
90	72	102	Pentobarbital, 40 mgm.
107	64	100	Heparin, 5 units
140	54	100	
165	60	98	Tremors
178	30	99	Convulsions

glomerular filtration following 1.0 mgm. per kgm. of NaFAc was quite small, while the concomitant reduction in PAH Tm was marked (fig. 1B). Following the administration of fluoroacetate the effects on PAH Tm usually appeared during the second hour following the administration of the inhibitor. From the data available, it is not possible to determine the relationship of this latency to the dosage of fluoroacetate injected since the variation from experiment to experiment was quite considerable. The changes in blood flow and glomerular filtration produced by NaFAc might have been a factor in the depression of PAH Tm. An attempt was thus made to correct for these changes and the renal load of PAH was calculated and plotted against PAH Tm (see methods). In fig. 2A, these data are given for some of the saline infusion experiments before and after 1.0 mgm. of NaFAc per kgm. of body weight. The post fluoroacetate data were

TABLE 2

The influence of sodium fluoroacetate on PAH Tm in the anesthetized dog during sodium chloride, sodium bicarbonate or sodium acetate infusions

All infusions contained p-aminohippurate, creatinine, sodium chloride, sodium acetate or sodium bicarbonate.

DOSE OF SODIUM FLUORO-ACETATE	GFR ML./MIN./M²		PLASMA PAH MGM. %		PAH Tm MGM./MIN./M²		PER CENT CHANGE IN PAH Tm	PER CENT CHANGE IN GFR	TYPE OF INFUSION
	Control	Exp.	Control	Exp.	Control	Exp.			
mgm./kgm.									
0.25	98	93	55.6	51.9	11.3	9.5	−16.0	−5.1	Infusion of isotonic saline 1.5–1.8 *mM* of NaCl per min. per m² of body surface
	60.3	54.9	59.6	68.1	12.1	10.2	−15.7	−8.1	
Av.							−15.8	−6.6	
0.5	88	64	47.1	69.2	11.6	3.85	−67.0	−23.3	
	98	73	55.6	59.0	11.3	5.1	−54.9	−25.5	
	60.3	40	59.6	84.0	12.5	5.0	−60.0	−32.3	
	79.8	44.8	53.4	66.8	8.9	1.2	−86.0	−43.9	
	79.5	57.2	51.4	53.3	11.2	4.0	−64.3	−28.1	
Av.							−66.5	−30.6	
1.0	88.	44.	47.1	84.6	11.6	0.70	−94	−50	
	83.	39.	127.0	137.6	7.2	1.20	−83.4	−53	
	70.0	65.4	49.5	54.8	9.7	0.70	−92.8	−6.6	
	77.5	36.2	48.8	65.4	15.1	2.00	−86.8	−53.3	
	79.5	42.9	51.4	65.0	11.2	0.70	−92.8	−6.6	
	79.8	32.3	53.4	83.5	8.9	0.30	−96.6	−58.9	
	68.0	61.	48.2	80.3	9.4	0.65	−93.1	−10.3	
	42.	28.	39.	48.8	22.5	10.80	−52.0	−33.4	
Av.							−86.6	−38.9	
2.0	83.	20.	127.	161.	7.2	−1.2	−100	−41	
	70	34.2	49.5	60.4	9.7	−.01	−98	−51.2	
Av.							−99	−46.1	
1.0	82.1	32.4	64.	89.8	9.4	0.4	−95.7	−60.6	Infusion of isotonic sodium bicarbonate 1.5–1.7 *mM* $NaHCO_3$ per min./m² of body surface
	94.0	41.5	42.1	59.8	10.1	0.6	−94.1	−53.9	
	72.2	18.5	53.2	79.8	14.7	1.6	−90.2	−74.4	
Av.							−93.3	−63.0	
1.0	76.8	32.4	49.0	56.3	19.	7.5	−60.5	−57.9	Infusion of isotonic sodium acetate 1.4-1.8 *mM* of NaAc/min. per m² of body surface
	64.0	27.0	43.2	96.3	20.8	6.9	−66.8	−58.	
	88.	68.2	44.4	45.2	22.1	9.9	−55.2	−22.5	
	92.8	30.5	59.3	67.8	21.6	12.4	−42.6	−67.2	
Av.							−56.3	−51.4	
1.0	108.	33.5	39.5	48.2	22.8	19.5	−14.5	−69.	Infusion of 3x isotonic sodium acetate 4.5–5.0 *mM* of NaAc per min./m² of body surface
	86.1	42.8	67.8	83.9	19.4	18.	−7.7	−50.3	
	94.5	40.0	47.5	68.4	23.6	18.	−23.7	−57.7	
	83.	23.6	60.4	89.6	21.5	17.5	−18.6	−71.6	
Av.							−16.1	−62.2	

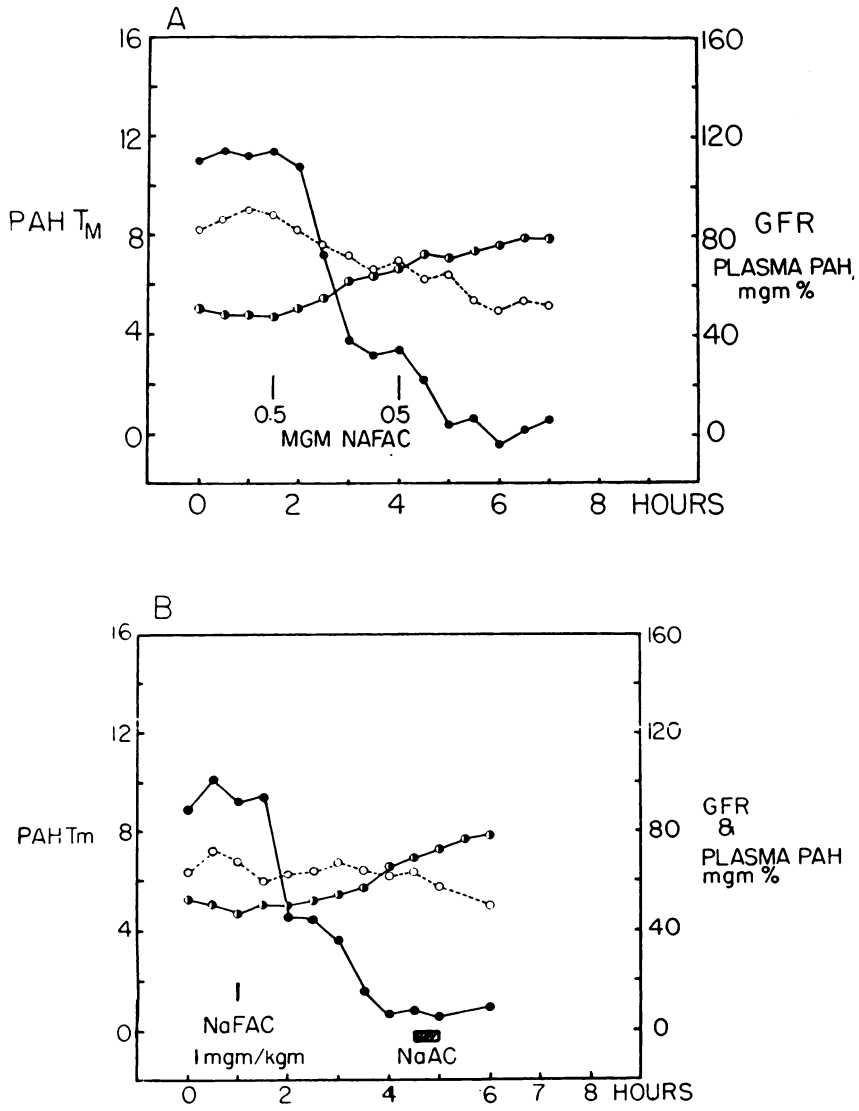

FIG. 1. The influence of sodium fluoroacetate on the glomerular filtration and PAH Tm in the dog. All values are per m^2 of body surface. Infusions consisted of isotonic saline containing creatinine and PAH.

A. Female dog, 15.2 kgm. Pentobarbital anesthesia. NaFAc: 0.5 mgm. of sodium fluoroacetate per kgm. given intravenously. Saline infusions about 10 ml. per min. per m^2.

B. Female dog, 13.6 kgm. Pentobarbital anesthesia. NaFAc: 1 mgm. of sodium fluoroacetate per kgm. given intravenously. Saline infusions about 10 ml. per min. per m^2.

NaAc: Continuous infusion of isotonic sodium acetate, 1.66 *mM* of sodium acetate per minute per m^2 of body surface.

● PAH Tm mgm. per min.
○ Glomerular filtration ml. per min.
◑ Plasma PAH concentration mgm. per cent

calculated from values obtained two hours or more after the injection of the inhibitor. From fig. 2A it can be seen that following fluoroacetate PAH Tm was reduced within a wide range of PAH load.

The influence of sodium bicarbonate on the effects of sodium fluoroacetate on PAH secretion was studied in three experiments. Isotonic sodium bicarbonate was infused at about 10 ml. per minute per m^2 of body surface and about one hour after the bicarbonate infusion was started, 1.0 mgm. of NaFAc per kgm. of body weight was injected. The results were comparable to those obtained with isotonic sodium chloride infusions (table 2, fig. 2A). When isotonic sodium ace-

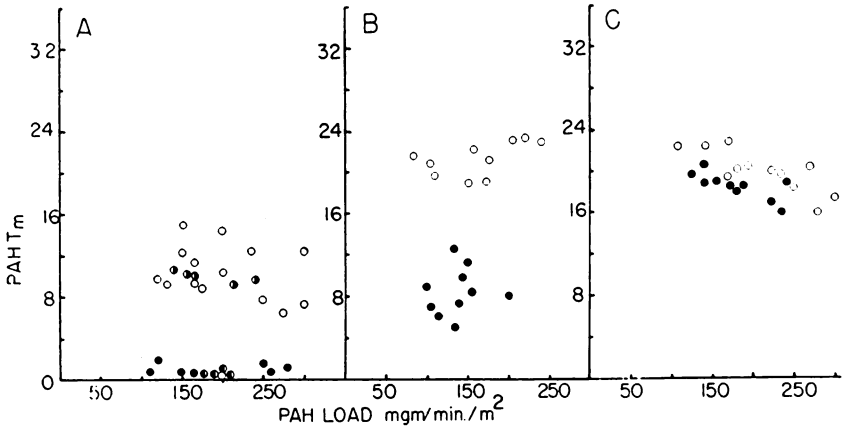

FIG. 2. The relationship of renal load of PAH to PAH Tm as affected by sodium fluoro acetate (1.0 mgm. per kgm. *i.v.*) and sodium acetate infusions.

A. Infusion of isotonic saline or sodium bicarbonate 1.5–1.8 *mM* per min. per m^2.
- ○ Saline infusions before NaFAc
- ● Saline infusions after NaFAc
- ◑ Bicarbonate infusion before NaFAc
- ◐ Bicarbonate infusion after NaFAc

B. Infusion of isotonic sodium acetate 1.4–1.8 *mM* of NaAc per min. per m^2.
- ○ Before NaFAc
- ● After NaFAc (1.0 mgm. per kgm.)

C. Infusion of 3x isotonic sodium acetate 4.5–5.0 *mM* of NaAc per min. per m^2.
- ○ Before NaFAc mgm per min.
- ● After NaFAc (1.0 mgm. per kgm.)

tate was infused instead of saline, PAH Tm during the control period was considerably higher than during the sodium chloride infusion. The injection of fluoroacetate still reduced glomerular filtration to about the same extent as in the saline experiments. PAH Tm was still reduced by fluoroacetate although the reduction was less than in the saline experiments (table 2). In fig. 2B, renal load of PAH was plotted against PAH Tm in a manner similar to fig. 2A. It can be seen that the substitution of isotonic sodium acetate for sodium chloride resulted in a partial protection of the kidney against fluoroacetate. In four dogs the concentration of acetate in the infusion was increased from 2.1 to 6.0 per cent and

about one hour after the start of the sodium acetate infusion 1.0 mgm. of NaFAc per kgm. of body weight was given intravenously. From table 2 and fig. 2C it can be seen that raising the acetate concentration in the infusion produced a nearly complete protection of the renal PAH transport mechanism although glomerular filtration was still markedly reduced by fluoroacetate.

When sodium acetate was infused about two hours after the administration of fluoroacetate the results obtained were quite different. Mudge and Taggart (1950) have shown that the intravenous administration of sodium acetate increased the PAH Tm in the dog. This observation has been confirmed. However,

TABLE 3

Effect of sodium fluoroacetate on the sodium acetate induced stimulation of PAH Tm

All experiments were conducted on anesthetized dogs. Values given are per one m^2 of body surface. All animals received isotonic saline infusions at a rate of about 10 ml. per minute per m^2 containing PAH, creatinine and sodium chloride or sodium acetate.

EXP.	GFR ML./MIN.			PLASMA PAH CONC. MGM. %			PAH Tm MGM./MIN.			REMARKS
	Control	After NaFAc	After NaAc	Control	After NaFAc	After NaAc	Control	After NaFAc	After NaAc	
1	69.4	—	72.0	68.0	—	49.3	9.8	—	15.8	Normal anesthetized dogs received infusions of isotonic NaAc 1.5–1.7 *mM* per m^2 per min.
2	78.5	—	61.8	96.5	—	74.3	11.2	—	24.5	
3	44	—	40.5	44.1	—	33.8	17.2	—	28.7	
1	83	49	30	127	137	144	7.2	1.2	0.6	Anesthetized dogs received 1.0 mgm. per kgm. of NaFAc; about 2 to 3 hours later 1.4–1.8 *mM* NaAc per m^2 per min. was infused.
2	68	61	44	48.2	80.3	107	9.4	0.65	0.95	
3	77.5	46.2	29.5	48.8	65.4	88	15.1	2.0	1.2	
4	42	28	20.5	39.0	48.8	61.0	22.5	10.8	8.3	
1	83	30	21	127	144	182	7.2	0.6	0.8	Anesthetized dogs received 1.0 mgm. per kgm. of NaFAc. About 3 hours later 3 ml. monoacetin per kgm. were given intramuscularly.
2	68	44	28	48.2	107	133	9.4	0.95	—	
3	42	20.5	18	39.0	61.0	79.5	22.5	8.3	6.4	

the prior administration of sodium fluoroacetate completely eliminated this stimulating effect of acetate on PAH Tm (table 3). Schachter and Freinkel (1951) have described the stimulation of a self depressed PAH Tm by sodium acetate. Figure 3 shows that also this effect of acetate is abolished by the prior administration of NaFAc. Chenoweth *et al.* (1952) have shown that monoacetin[3] in a dosage of 3 to 5 ml. per kgm. given intramuscularly could reverse some of the cardiac and central nervous system effects of sodium fluoroacetate. In three experiments

[3] Monoacetin was kindly supplied by Dr. M. B. Chenoweth, Department of Pharmacology, Ann Arbor, Michigan.

monoacetin in the above dosage did not reverse the fluoroacetate inhibited PAH Tm although the muscle tremors and convulsive movements were markedly reduced by this dosage of monoacetin (table 3).

Effects of fluoroacetate on glucose Tm. The administration of 1 mgm. of fluoroacetate per kgm. of body weight resulted in a reduction of glucose Tm. This reduction started about 60 to 120 minutes after the fluoroacetate administration and reached its maximum about three hours after the injection of this inhibitor. Attempts to determine the effects of fluoroacetate on PAH Tm and glucose Tm simultaneously were not possible since in the presence of the high concentration

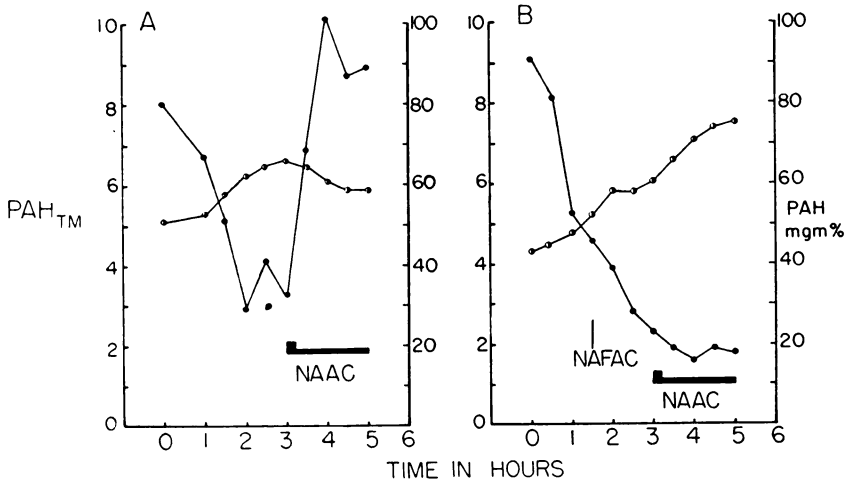

FIG. 3. The effect of sodium acetate infusion on the self depressed PAH Tm in the normal and in the sodium fluoroacetate poisoned dog. Anesthesia, pentobarbital.

A. Normal dog: NaAc: infusion of 4 *mM* of isotonic sodium acetate followed by 1.5 *mM* per minute per m² of body surface.

B. NaFAc: 1 mgm. of sodium fluoroacetate per kgm. given intravenously.

NaAc: Infusion of sodium acetate same as in experiment A.

○ PAH Tm

◑ Plasma PAH concentration mgm. per cent

Abscissa: Time in hours; ordinate left hand side PAH Tm in mgm. per min. per m², right hand side plasma PAH concentration mgm. per cent.

of PAH glucose Tm was markedly depressed (two experiments). We have thus compared the effects of fluoroacetate on glucose Tm and on PAH secretion at low PAH plasma concentrations. Table 4 gives the results obtained in five experiments. It can be seen that both glucose Tm, PAH secretion and glomerular filtration are reduced by sodium fluoroacetate when saline was infused. When 3 per cent sodium acetate was substituted for the saline, fluoroacetate had practically no effect on PAH secretion, while glucose Tm and glomerular filtration were still markedly reduced (table 4, fig. 4).

Self depression of PAH Tm and the hemodynamic changes produced by NaFAc could in themselves modify PAH Tm. This possibility prompted us to

TABLE 4

The effect of 1 mgm. of sodium fluoroacetate per kgm. body weight on renal PAH secretion and glucose reabsorption in the anesthetized dog

About 3 to 4 half hour control periods were determined followed by the intravenous injection of 1.0 mgm. of NaFAc per kgm. body weight. The changes in glucose Tm and PAH secreted at about 3 hours after the NaFAc administration were used in this table. All infused solutions contained PAH glucose, creatinine, sodium chloride or sodium acetate.

GFR ML./MIN./M²		PLASMA PAH MGM. %		PLASMA GLUCOSE MGM. %		PAH SECRETED MGM./MIN./M²		GLUCOSE Tm MGM./MIN./M²		PER CENT CHANGE IN PAH SECRETED	PER CENT CHANGE IN GLUCOSE Tm	PER CENT CHANGE IN GFR	REMARKS
Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.				
86	24	1.92	3.78	836	1345	1.99	0.55	225	37	72.4	83.6	72.2	Infusion of isotonic saline; about 1.5 *mM* NaCl per min. per m² of body surface was infused
74.5	29.3	2.11	4.95	449	952	2.45	0.71	285	70.8	71.0	75.1	60.7	
57.6	19.6	1.65	5.08	464	808	1.98	0.64	220	60.5	67.8	72.3	66.0	
107	29.5	2.06	5.43	485	972	2.18	0.42	159	60.6	80.7	61.6	72.4	
92	22	1.55	3.35	512	944	1.29	0.605	194	60.8	53.5	68.6	76.0	
Average										69.1	72.2	69.4	
73.6	18	1.43	2.50	998	1830	2.61	2.29	290	118	12.3	59.3	75.5	Infusion isotonic sodium acetate; about 1.5 *mM* of NaAc per min. per m² of body surface was infused
69.4	33.4	1.18	2.91	848	1510	3.36	2.99	252	72	11.1	71.4	51.9	
75.8	15.4	1.74	2.05	730	1322	3.96	3.42	244	42	13.6	82.8	79.7	
57.8	17.0	1.63	1.88	654	988	2.25	2.20	250	45	2.3	82.0	70.6	
Average										9.8	73.9	66.4	

try an approach which would minimize the effects of the above changes. Cross and Taggart (1950) have described an elegant *in vitro* method for the determination of the PAH concentrating ability of rabbit kidney slices and this ability to concentrate PAH is probably the same as secretory activity (Mudge and Taggart, 1950). In anesthetized dogs the left kidney was first removed and the slice medium ratio (S:M ratio) of PAH was determined by the above method. Three hours later the second kidney was removed and the S:M ratio was determined on slices from this second kidney. Three control experiments were conducted in

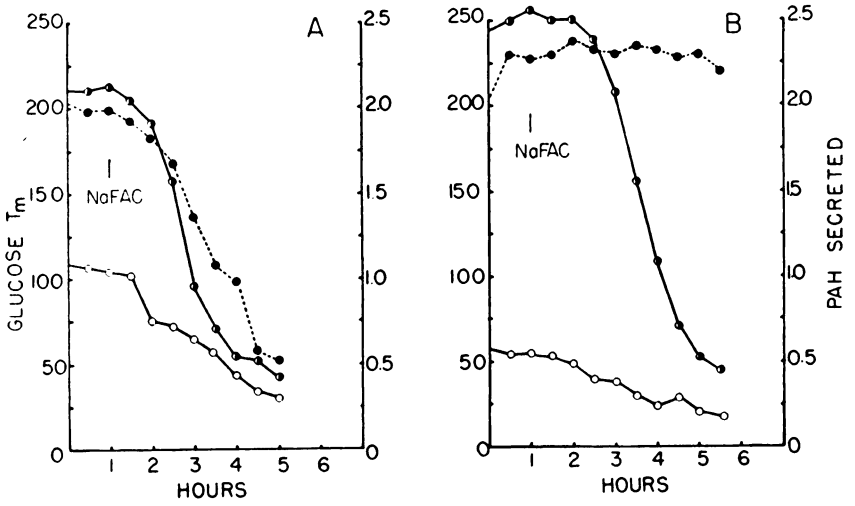

FIG. 4. The influence of fluoroacetate on glucose Tm and PAH secretion

A. Female dog, 10.3 kgm.: isotonic saline infusion about 1.4 *mM* of NaCl per min. per m^2. NaFAc: 1.0 mgm. of sodium fluoroacetate per kgm.

B. Female dog, 11.0 kgm.: isotonic sodium acetate infusion 1.58 *mM* of sodium acetate per min. per m^2.

NaFAc: 1.0 mgm. of sodium fluoroacetate *i.v.* per kgm. of body weight.

● PAH secreted
◑ Glucose Tm
○ Glomerular filtration

Abscissa: Time in hours; ordinate left hand side glucose Tm in mgm. per min. per m^2 and glomerular filtration ml. per min. per m^2 of body surface. Ordinate right hand side PAH secreted in mgm. per min. per m^2 of body surface.

this manner and the results are presented in table 5. It can be concluded that in the presence and absence of substrate the PAH S:M ratio for both right and left kidney are about the same. In another series of experiments a similar procedure was followed except that fluoroacetate was injected intravenously immediately after the removal of the first kidney and the results are presented in table 5. It can be seen that 1.0 to 2.0 mgm. of sodium fluoroacetate per kgm. of body weight had an effect on the PAH S:M ratio in the absence of sodium acetate as substrate. Acetate stimulation was marked in the unpoisoned slices but was completely absent in the fluoroacetate poisoned renal slices. It can be seen that even

after the highest dosage of fluoroacetate employed the kidney slices still showed a residual PAH concentrating ability both in the presence or absence of acetate as substrate.

DISCUSSION. The results presented show that fluoroacetate depressed the PAH Tm, glucose Tm, renal blood flow and glomerular filtration in the anesthetized dog. The fact that NaFAc produced hemodynamic changes made the interpretation of our results on tubular transport mechanism rather difficult. The attempts made to resolve this question were to relate the renal load of PAH to PAH Tm. By this method it was possible to show that PAH transport was significantly depressed by fluoroacetate. A further factor in favor of this interpretation is the fact that acetate infusions protect PAH Tm while similar infusions of acetate did not protect either the glomerular filtration on glucose Tm. This

TABLE 5

The effect of NaFAc on the PAH slice:medium ratio (SM ratio) of dog kidney slices

In all experiments the left kidney was removed first and the PAH SM ratio determined in the absence and presence of sodium acetate as substrate. Isotonic saline infusions were now started (about 10 ml. per min. per m^2 of body surface) and about 30 min. after the start of the infusion the indicated dose of NaFAc was injected intravenously. About 3 hours after the fluoroacetate injection the left kidney was removed and the PAH SM ratio was determined. In the first 3 animals depicted in this table no fluoroacetate was given showing that the SM ratio is not significantly changed by the saline infusion.

DOSAGE OF NaFAc MGM./KGM.	SM RATIO (LEFT KIDNEY) CONTROL		SM RATIO (RIGHT KIDNEY) EXPERIMENTAL	
	No substrate	Sodium acetate 0.01*M*	No substrate	Sodium acetate 0.01*M*
none	3.8	10.8	4.0	10.3
none	4.4	12.4	3.9	11.6
none	3.2	11.4	3.6	12.3
1 mgm.	3.9	10.6	2.6	2.3
1 mgm.	4.3	12.1	2.0	1.8
1 mgm.	3.2	9.2	2.3	2.0
1.5 mgm.	4.8	14.5	2.0	1.8
2 mgm.	4.0	11.8	1.7	1.6

fact makes it clear that the fluoroacetate induced reduction in glomerular filtration did not influence PAH Tm to any great extent and it is thus probable that the effects of NaFAc on PAH Tm are due to an interference of fluoroacetate with the renal cellular transport of PAH. Further evidence in favor of this mechanism are the data presented in table 5 which show that renal slices obtained from fluoroacetate poisoned dogs show a reduction in their ability to concentrate PAH both in the absence and presence of sodium acetate as substrate.

The effects of fluoroacetate on glucose Tm could not be prevented while PAH secretion was definitely protected by sodium acetate infusions (table 4, fig. 4). Glomerular filtration was reduced by fluoroacetate both in the absence and presence of acetate. It must be concluded that as far as PAH secretion, the changes in glomerular filtration observed did not appreciably influence the amount of

PAH secreted and the effects of fluoroacetate were due to an interference with the tubular secretory mechanism for PAH.

The effects of fluoroacetate on glucose Tm are not as clearly demonstrable. The changes in filtration rate could explain the reduction in glucose Tm observed. However, Thompson, Barrett and Pitts (1951) have shown that a reduction in glomerular filtration rate of 60–70 per cent resulted in a 30 per cent reduction of glucose Tm. We have conducted two experiments where glomerular filtration and renal blood flow were reduced by hemorrhages. One of these

TABLE 6

The reaction of glomerular filtration, PAH secretion and glucose Tm to hemorrhage

Female dog, 11.5 kgm. Pentobarbital anesthesia. Infusion of 10 per cent glucose and 0.2 per cent NaCl at a rate of 10.3 ml. per min. per m^2 of body surface.

TIME, MIN.	GFR	PLASMA CONC.		GLUCOSE Tm	PAH SECRETED
		PAH mgm. %	Glucose mgm. %		
0–30	79.7	1.51	500	174	2.65
30–60	75.6	1.69	540	156	2.57
60—Bled 120 ml.					
60–120	58.6	2.32	720	160	2.68
120—Bled 60 ml.					
120–180	51.0	2.71	930	121	2.66
180—Bled 90 ml.					
180–210	43.1	2.80	1040	109	2.42
210–270	43.6	2.93	1204	140	2.76
270–285	38.6	2.98	1242	118	2.80
285—Bled 90 ml.					
285–300	31.6	3.06	1292	90	2.78
300–315	34.9	3.07	1350	130	3.08
315—Bled 90 ml.					
315–330	24.2	3.18	1423	101	2.49
330—Bled 60 ml.					
330–345	20.3	3.37	1500	114	2.35
345—Bled 60 ml.					
345–360	9.10	3.82	1660	25.0	1.49
360—Bled 60 ml.					
360–390	8.7	4.33	1870	30.0	1.78

experiments is given in table 6 and it is apparent that a reduction of about 70–75 per cent in glomerular filtration resulted in approximately 35 per cent reduction in glucose Tm. The reduction in glucose Tm produced by fluoroacetate at comparable reductions of glomerular filtration were always higher. It is thus likely that fluoroacetate does interfere with tubular glucose reabsorption to some extent; however, the data available do not allow any quantitative conclusions.

It is of interest to note that dinitrophenol (Mudge and Taggart, 1950) in dosages which have a profound effect on PAH Tm does not influence glucose Tm. In a similar manner it was shown that triacetin in dosages which produced a

depression of PAH Tm did not appreciably effect glucose Tm (Shideman *et al.* 1952). These differences between fluoroacetate, dinitrophenol and monoacetin may simply be a question of dosage and it is possible that larger amounts of the latter inhibitors could conceivably depress glucose reabsorption. Mudge and Taggart (1950) have not been able to demonstrate any effects of dinitrophenol on electrolyte excretion. With fluoroacetate it is possible to show changes on renal acid, sodium and chloride excretion (Farah, Graham and Koda, 1953). It is thus likely that renal effects of fluoroacetate are less specific than those produced by dinitrophenol. The possibility of more than one site of action of NaFAc must be kept in mind (Bush and Potter, 1952), thus explaining the differential effects of acetate infusions on the fluoroacetate induced blocking of glucose and PAH renal transport. From the evidence presented it is possible that fluoroacetate acts competitively with acetate on the PAH transport mechanism while it may act noncompetitively on renal tubular glucose reabsorption.

SUMMARY

In anesthetized dogs sodium fluoroacetate (NaFAc) reduced renal blood flow, para-aminohippurate, (R.P.F.), creatinine and inulin clearance (GFR) as well as para-aminohippurate and glucose transport maximum (PAH Tm, glucose Tm). Infusions of sodium acetate increased PAH Tm and protected against the effects of sodium fluoroacetate on the PAH secretory mechanism. Once fluoroacetate effects had occurred acetate had no stimulatory effect on PAH Tm. The evidence presented suggests a competitive mechanism between NaFAc and sodium acetate on the PAH secretory mechanism. Kidney slices from fluoroacetate poisoned dogs show a reduction in their ability to concentrate PAH both in the absence and presence of sodium acetate as substrate.

Glucose Tm is reduced by fluoroacetate administration. It differs from the PAH secretory mechanism in that sodium acetate does not protect the glucose reabsorptive mechanism against sodium fluoroacetate effects. The renal hemodynamic changes produced by NaFAc make it difficult to interpret these effects on glucose Tm. It is possible that a part of the fluoroacetate induced reduction in glucose Tm is due to a blocking of renal tubular reabsorption of glucose.

REFERENCES

Buffa, P., and Peters, R. A.: J. Physiol., **110:** 488, 1949.
Buffa, P., Peters, R. A., and Wakelin, R. W.: Biochem. J., **48:** 467, 1951.
Busch, H., and Potter, V. R.: Proc. Soc. Exp. Biol. & Med., **80:** 70, 1952.
Chenoweth, M. B.: Pharmacol. Rev., **1:** 383, 1949.
Chenoweth, M. B., Kandel, A., Johnson, L. B., and Bennett, D. R.: This Journal, **101:** 31, 1951.
Cross, R. J., and Taggart, J. V.: Am. J. Physiol., **161:** 191, 1950.
Dumke, P. R., and Schmidt, C. F.: Am. J. Physiol., **138:** 421, 1942–43.
Farah, A., Graham, G., and Koda, F.: Unpublished results, 1953.
Folin, O., and Wu, H.: J. Biol. Chem., **38:** 81, 1919.
Graham, G., and Farah, A.: Fed. Proc., **11:** 350, 1952.
Himwich, H. E.: Quoted from Chenoweth, M. B., Pharmacol. Rev., **1:** 383, 1949.
Kandel, A., Johnson, L. B., and Chenoweth, M. B.: Fed. Proc., **10:** 312, 1951.

LIEBECQ, C., AND PETERS, R. A.: J. Physiol., **108:** 11P, 1948.
LOTSPEICH, W. D., PETERS, R. A., AND WILSON, T. H.: Biochem. J., **48:** 467, 1951.
MARTIUS, C.: Liebigs Ann., **561:** 227, 1949.
MUDGE, G. H., AND TAGGART, J. V.: Am. J. Physiol., **161:** 191, 1950.
NELSON, N.: J. Biol. Chem., **153:** 375, 1944.
POTTER, V. R., AND BUSCH, H.: Fed. Proc., **9:** 215, 1950.
SCHACHTER, D., AND FREINKEL, N.: Am. J. Physiol., **167:** 531, 1951.
SCHREINER, G. E.: Proc. Soc. Exp. Biol. & Med., **74:** 117, 1950.
SHIDEMAN, F. E., AND RENE, R. M.: Am. J. Physiol., **166:** 104, 1951.
SHIDEMAN, F. E., WOODS, L. A., AND SEEVERS, M. H.: THIS JOURNAL, **101:** 31, 1951.
SMITH, H. W., FINKELSTEIN, N., ALIMINOSA, L., CRAWFORD, B., AND GRABER, M.: J. Clin. Invest., **24:** 388, 1945.
TAGGART, J. V.: Am. J. Physiol., **167:** 248, 1951.
THOMPSON, D. D., BARRETT, M. J., AND PITTS, R. F.: Am. J. Physiol., **167:** 546, 1951.