Direct Effects of Salicylate on Renal Function in the Dog

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ABSTRACT Sodium salicylate was administered to anesthetized dogs in doses sufficient to produce concentrations in plasma comparable to those common in human salicylate toxicity. Salicylate administration increased the rates of excretion of water, sodium, and chloride in the urine. Salicylate administration also increased the rate of excretion of potassium so that its clearance often exceeded that of creatinine. This enhancement of potassium excretion was dissociated from the alkalosis that accompanies salicyate toxicity. Administration of 5% CO_2 in inspired gas did not attenuate the excretion of potassium; injection of salicylate into one renal artery caused a unilateral kaliuresis. Phosphate excretion increased progressively after administration of salicylate. On several occasions the clearance of phosphate equalled that of creatinine. Salicylate reduced renal tubular glucose reabsorption. When salicylate was injected into a renal artery, a glycosuria occurred ipsilaterally at filtered loads of glucose far below the reabsorptive capacity of the dog kidney. Salicylate administration also was associated with early elevation of glucose, phosphate, and potassium concentration in plasma. Salicylate administration reduced the content of adenosine triphosphate in the renal medulla. Salicylate was concentrated within the medulla between 1.5 and 3 times that of the cortex, a gradient equal to that for chloride.

INTRODUCTION

Alterations in acid-base balance in salicylate poisoning are severe and common. The most immediate disturbance in adults is respiratory alkalosis. In children, and late in the course of salicylate poisoning in adults, the dominant change is metabolic acidosis. The acidosis probably is due to accumulation of lactic and other organic acids (1). Reports of other alterations in composition of extracellular fluid are conflicting. The concentration of potassium in plasma has been reported as increased (2), decreased (3), or unchanged (4, 5). Hyperglycemia (6) as well as hypoglycemia (7) is described. Values of plasma phosphate concentration have been reported as unchanged (4), and the occasional observation of elevated phosphate was attributed to prerenal azotemia (8). Dehydration is a prominent feature of salicylate toxicity and has been attributed to vomiting, fever, sweating, and lack of adequate fluid intake, but not to diuresis.

References to the effects of salicylate on renal excretion of salt and water are few and the results are variable. Polyuria has been noted (8), although oliguria has also been described (6, 9). Sodium excretion has been reported unchanged (4), decreased (10, 11), and increased (12). Observations on phosphate excretion also are controversial (4, 11, 12). Such discrepancies may be explained by species differences and by differences in dose, duration of observation and other experimental variables. It is generally assumed that potassium loss is secondary to respiratory alkalosis (8, 9, 13). Only one report, in abstract form, has challenged this viewpoint (14).

The present report describes the acute effects of toxic doses of salicylate administered systemically and by renal artery infusion on renal function in the anesthetized dog. Salicylate caused a natriuresis associated with chloruresis and diuresis in confirmation of the findings of Ramsay and Elliott (12). Unilateral administration of the compound produced a unilateral kaliuresis during respiratory acidosis caused by inspiration of 5% CO_2. Glycosuria and phosphaturia were noted without alteration in the filtered loads of these substances.

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In addition, we found in experimental animals that salicylate accumulated in the renal medulla to an extent approximating the gradient for chloride. Salicylate also reduced the concentration of ATP in the renal cortex.

METHODS

Two types of experiments were performed in fasting dogs, weighing 15–20 kg, lightly anesthetized with pentobarbital. In six experiments salicylate was injected intravenously; in seven experiments salicylate was injected directly into one renal artery.

Experiments with i.v. injection. The dogs were given approximately 1000 ml isotonic saline solution i.v. in 1 h followed by a continuous infusion of isotonic saline solution containing 5 g of creatinine per liter, at a rate of 5 ml/min. Urine was collected from each kidney by direct ureteral catheterization. Samples of blood were taken from a femoral artery. Urine samples were collected only when rates of urine flow were approximately equal in both kidneys. Two control collections were obtained from each kidney at 10-min intervals. Typically, the interval between onset of fluid administration and initiation of clearance collections was 2 h. Control periods were followed by administration of sodium salicylate (Sigma, reagent grade) dissolved in 50 ml of water, injected intravenously over 5 min in a dose of 3 mmol/kg body weight. Subsequent collections were made at 10-min intervals for 60–80 min.

To ascertain that the results were not due to volume expansion, a control series of four experiments were carried out under identical conditions, except that sodium chloride (Baker, reagent grade) in a dose of 3 mmol/kg body weight was injected intravenously instead of salicylate.

Experiments with intraarterial infusion. In seven experiments the left kidney was approached retroperitoneally by an incision in the flank with blunt dissection of the underlying muscles. A small, curved needle was inserted into the left renal artery which was being perfused at 1.38 ml/min with an isotonic saline solution. The experimental preparation was otherwise similar to the one used for i.v. injection. After two control clearance periods, a 0.36 M solution of sodium salicylate was infused directly into the renal artery at the rate of 1.38 ml/min (0.5 mmol/min). Experimental observations were continued for 60–80 min of salicylate infusion. We also performed four additional control studies in which the experimental conditions were identical with the above except that a 0.36 M solution (0.5 mmol/min) of sodium chloride was injected instead of salicylate.

Upon completion of an experiment, the left kidney was exteriorized through the flank incision and sliced with a three-bladed knife. The slices were dropped into liquid nitrogen, which freezes the tissue within 8 s of interruption of its blood supply (15, 16). Frozen samples of cortex and inner medulla were separated and their content of adenine nucleotides measured by a modification (16) of the Cohen and Carter method (17). In six experiments, the concentration of salicylate and chloride was measured in cortex, outer medulla, and inner medulla after chipping off portions of tissue of these zones from frozen slices. The tissues were ground in the cold, weighed, and let stand overnight in 5 times their weight of water at 4°C (18). These specimens were centrifuged and decanted, and the supernatant was analyzed for salicylate and chloride content.

Creatinine was measured by the method of Jaffe as modified by Smith (19), PAH by the method of Smith et al. (20), and inorganic phosphate by the method of Chen et al. (21). Salicylate was measured by the method of Brodie et al. (22) and glucose by the glucosoxidase method (23). Sodium and potassium were determined by flame photometry and chloride by the Cotlove chloridometer.

RESULTS

Systemic infusion

The results of six experiments in which salicylate was injected intravenously are presented in Table I. Data of two consecutive control periods are presented from minus 20 min to zero time. Salicylate was infused during 5 min and samples of three to seven consecutive experimental periods were collected subsequent to its administration.

Salicylate induced a prompt and marked diuresis associated with an increase in the excretion rates of sodium, potassium, phosphate, and chloride. Creatinine clearance remained essentially unchanged. Plasma concentrations of potassium and phosphate increased in every experiment. Mean urine volume was 0.95 ml/min during control periods and 7.44 ml/min during the last period of salicylate administration. Mean sodium clearance was 0.88 ml/min during control periods and 7.66 ml/min during the last experimental period. Mean clearance of potassium was 4.8 ml/min before salicylate and 38.1 ml/min at the end of the experiment. In one example of this series, potassium clearance exceeded creatinine clearance. Phosphate clearance was 5.03 ml/min during the control periods and 33.4 ml/min during the last period.

Clearance of chloride, measured in four experiments, paralleled sodium clearance. Plasma glucose concentration increased in every experiment. Peak concentration was reached soon after injection of salicylate and, by end of the experiment, the level characteristically was declining. In one experiment (no. 30) the plasma glucose concentration fell below control values. Glucosuria occurred in only two experiments of this series. In both, hyperglycemia was present at the time of glucosuria.

The results of the four control experiments are presented in Table II. Two control clearance period collections of 10 min duration preceded the administration of 3 mmol of sodium chloride. This was followed by collection of samples of four or five experimental periods. There was a slight increase in the rate of urine flow and electrolyte excretion immediately after the injection of sodium chloride, but this effect was short-lived. After 20 or 30 min the clearance of phosphate, sodium, and potassium did not differ significantly from the controls.

In eight additional experiments, in which salicylate was given i.v., observations were prolonged up to 200 min. In these experiments the initial elevation of phosphate, potassium, and glucose in plasma was followed in

TABLE I

Systemic Injection of Salicylate, Data of Left Kidney

Experiment no.	Time	V	C_{Cr}	P_{PO_4}	C_{PO_4}	P_{Na}	C_{Na}	P_K	C_K	P_G
	min	*ml/min*	*ml/min*	*μmol/ml*	*ml/min*	*μeq/ml*	*ml/min*	*μeq/ml*	*ml/min*	*mg/ml*
25	−20–10	0.2	37.7	0.86	5.3	158	0.19	4.0	1.5	1.05
	−10–0	0.1	40.1	0.92	4.0	156	0.15	4.0	1.4	—
	0–5				+Salicylate, 3 mmol/kg					
	5–15	3.8	32.8	1.02	19.6	162	3.80	5.9	16.2	—
	15–25	3.5	37.7	1.25	28.9	161	3.80	5.4	24.8	1.27
	25–35	3.9	36.9	1.39	31.1	157	3.90	5.1	29.4	1.23
	35–45	4.1	36.7	1.52	30.2	158	3.80	5.3	29.1	1.10
	45–55	5.0	36.5	1.59	30.0	156	4.50	5.2	33.0	1.10
	55–65	5.5	32.5	1.48	28.7	156	4.80	4.8	37.1	1.17
26	−20–10	0.5	39.7	0.77	1.90	146	0.83	3.3	1.1	1.39
	−10–0	0.5	33.2	0.72	0.70	145	0.81	3.2	1.0	1.37
	0–5				+Salicylate, 3 mmol/kg					
	5–15	7.4	38.2	1.09	28.8	152	7.88	4.1	26.0	1.95
	15–25	7.2	40.6	1.43	34.6	149	8.06	4.1	31.1	1.82
	25–35	7.6	42.6	1.34	37.0	150	8.40	3.9	37.4	1.76
27	−20–10	0.2	38.0	0.95	3.0	141	0.17	3.9	3.6	1.14
	−10–0	0.2	40.6	—	—	141	0.15	4.0	3.5	1.11
	0–5				+Salicylate, 3 mmol/kg					
	5–15	7.7	41.0	1.17	28.8	143	7.86	4.9	35.0	1.44
	15–25	4.1	43.1	1.17	28.0	145	4.89	4.1	36.5	1.38
	25–35	5.1	48.1	1.42	27.5	145	5.88	4.0	38.3	1.40
	35–45	6.0	47.7	1.86	32.1	144	6.65	4.2	39.4	1.36
28	−20–10	0.8	47.0	1.34	5.4	151	1.27	3.7	7.5	1.06
	−10–0	0.7	40.4	1.35	5.0	149	1.17	3.7	7.4	1.06
	0–5				+Salicylate, 3 mmol/kg					
	5–15	6.4	39.0	1.83	28.1	153	7.70	5.2	28.4	1.75
	15–25	4.8	42.0	2.04	32.8	151	6.12	5.3	31.7	1.56
	25–35	4.5	42.0	1.97	34.2	149	5.77	5.4	34.3	1.56
	35–45	4.7	44.1	1.84	35.8	147	5.85	5.3	37.6	1.48
	45–55	5.3	43.2	1.76	36.1	149	6.45	5.3	40.2	1.43
30	−10–0	2.3	31.9	1.60	8.0	145	2.3	3.2	8.6	1.02
	0–5				+Salicylate, 3 mmol/kg					
	5–15	7.1	30.8	1.60	15.8	146	7.2	4.3	24.6	1.23
	15–25	5.8	34.1	1.83	20.5	146	6.0	4.2	25.7	1.22
	25–35	6.0	31.7	2.14	22.6	147	5.7	4.5	25.3	0.98
	35–45	6.5	32.3	2.36	23.1	148	5.8	4.7	25.9	0.99
	45–55	8.8	32.3	2.50	26.6	149	8.1	5.3	27.5	1.02
	55–65	11.0	33.6	2.46	26.5	149	10.0	6.0	28.7	0.85
	65–75	11.9	33.2	2.21	28.3	149	11.1	6.6	29.4	0.78
31	−20–10	0.7	35.7	1.17	9.6	141	0.9	3.2	7.5	0.95
	−10–0	0.4	33.9	1.17	8.2	140	0.4	3.2	6.1	1.05
	0–5				+Salicylate, 3 mmol/kg					
	5–15	6.5	41.3	2.00	32.5	141	7.0	4.3	29.9	1.25
	15–25	5.0	45.0	1.65	35.0	143	5.5	4.5	37.5	1.40
	25–35	4.6	42.4	1.65	35.9	145	5.0	4.7	37.5	1.42
	35–45	8.5	45.2	1.65	39.4	143	8.6	4.8	44.6	1.42
Mean ±SEM										
Controls		0.95±0.32	37.5±1.7	1.11±0.13	5.03±1.2	147±2	0.88±0.32	3.53±0.15	4.8±1.3	1.11±0.06
Last Period		7.44±1.02	40.7±2.6	1.72±0.12	33.4±2.0	148±2	7.66±0.88	4.93±0.39	38.1±2.0	1.32±0.13

Data of each control and experimental period in six experiments. In this and in the following tables means and standard errors of the mean for controls refer to an average of the two control periods.

several instances by a late decline to concentrations equal to or below those observed during control periods (Fig. 1). Potassium and phosphate clearances were elevated throughout these experiments.

TABLE II

Systemic Injection of Sodium Chloride, Data of Left Kidney

Experiment no.	Time	V	C_{Cr}	P_{PO_4}	C_{PO_4}	P_{Na}	C_{Na}	P_K	C_K
		ml/min	*ml/min*	*μmol/ml*	*ml/min*	*μeq/ml*	*ml/min*	*μeq/ml*	*ml/min*
1	−20–10	1.25	25.4	1.46	10.7	144	1.23	3.6	5.59
	−10–0	1.35	23.9	1.56	11.4	144	1.30	3.5	6.21
	0–5			+Sodium chloride, 3 mmol/kg					
	5–15	1.61	28.9	1.51	14.4	144	1.91	3.5	8.97
	15–25	1.74	28.4	1.56	16.4	146	2.03	3.7	10.20
	25–35	1.90	28.2	1.61	16.9	144	2.30	3.6	11.19
	35–45	1.80	29.8	1.54	18.1	144	2.18	3.6	9.70
2	−20–10	0.20	44.5	1.51	4.8	143	0.11	3.9	3.35
	−10–0	0.20	48.3	1.51	4.1	143	0.08	3.7	2.33
	0–5			+Sodium chloride, 3 mmol/kg					
	5–15	0.60	55.3	1.39	18.3	150	0.49	3.7	8.92
	15–25	0.25	30.6	1.03	6.0	147	0.30	3.9	2.47
	25–35	0.26	30.6	1.66	9.9	150	0.18	4.2	4.27
	35–45	0.22	30.6	1.67	8.3	146	0.14	4.1	3.84
3	−20–10	2.70	39.0	1.20	10.3	144	3.10	3.4	13.9
	−10–0	3.10	39.3	1.34	9.8	144	3.42	3.4	15.4
	0–5			+Sodium chloride, 3 mmol/kg					
	5–15	3.90	38.7	1.32	12.5	143	4.69	3.3	18.7
	15–25	3.70	40.2	1.34	12.6	144	4.50	3.3	19.6
	25–35	3.64	39.6	1.35	12.6	146	4.41	3.3	19.9
	35–45	3.58	32.4	1.35	10.8	146	4.39	3.4	19.5
	45–55	3.28	38.8	1.14	13.4	145	4.09	3.6	17.2
4	−20–10	2.55	37.8	0.94	6.5	146	1.96	2.9	10.82
	−10–10	3.25	39.4	0.96	7.2	147	2.54	2.9	14.32
	0–5			+Sodium chloride, 3 mmol/kg					
	5–15	6.15	40.9	1.00	10.1	151	5.01	2.9	17.18
	15–25	5.20	41.5	1.01	11.2	149	4.40	3.0	16.99
	25–35	2.90	36.2	1.07	8.8	151	2.48	3.0	13.44
	35–45	2.80	42.0	1.09	8.9	149	2.33	3.4	9.70
Mean ±SEM	Controls	1.83±0.66	37.2±4.5	1.31±0.13	9.81±1.9	144±1	1.73±0.68	3.4±0.2	8.99±2.77
	Last period	2.03±0.68	35.3±3.0	1.36±0.14	12.2±2.3	146±1	2.18±0.81	3.7±0.1	10.11±2.74

Data of left kidney in six experiments. After two control periods, sodium chloride, 3 mmol/kg, was injected intravenously.

Toward the end of these observations the ratio of potassium clearance to creatinine clearance varied from 1.05 to 2.0 (Fig. 2). This is evidence for tubular secretion of potassium. In two experiments the dogs inhaled 5% CO_2 in air. This maneuver, which produces a moderate respiratory acidosis, did not alter the kaliuresis. Potassium clearance in these two experiments was 8.6 and 6.7 ml/min before administration of salicylate and increased to 29.4 and 44.6 ml/min, respectively, during infusion of salicylate despite inhalation of CO_2.

Intraarterial infusion

Data of seven experiments in which salicylate was injected unilaterally are presented in Table III. Samples of two 10-min clearance periods were collected as controls. Direct injection into the renal artery permitted the use of smaller doses of salicylate than those employed by intravenous route.

In every instance, a diuresis was produced exclusively on the injected side. Mean urinary volume on the left

side increased from 1.90 ml/min during the control periods to 5.40 ml/min during the last experimental period. Water excretion on the contralateral side was unchanged or decreased. On the experimental side, creatinine clearance declined slightly in some experiments and, in one case, increased modestly during the last experimental period. Phosphate clearances increased in both kidneys but predominantly in the infused side. The mean C_{PO_4} in the left kidney during control periods was 9.8 ml/min and 23.1 in the last period; in the right side the increase was from 8.7 to 14.8 ml/min.

Sodium clearance changed only from 2.35 to 2.47 ml/min on the right side, while on the experimental side it increased from 2.64 to 5.89 ml/min. Potassium clearance increased on the experimental side in every experiment and bilaterally in three of seven studies. Mean potassium clearance on the right side increased from 9.3 to 18.6 ml/min and from 9.8 to 27.4 ml/min in the left side. The rates of excretion of sodium and potassium in one such experiment are shown graphically in Fig. 3.

The results of four control experiments with sodium chloride are presented in Table IV. After two control periods sodium chloride was infused into the renal artery at a molar rate equal to or higher than salicylate. This infusion did not cause any significant enhancement in the excretion of sodium, potassium, or phosphate in the injected side in contrast with the effects produced by infusion of salicylate.

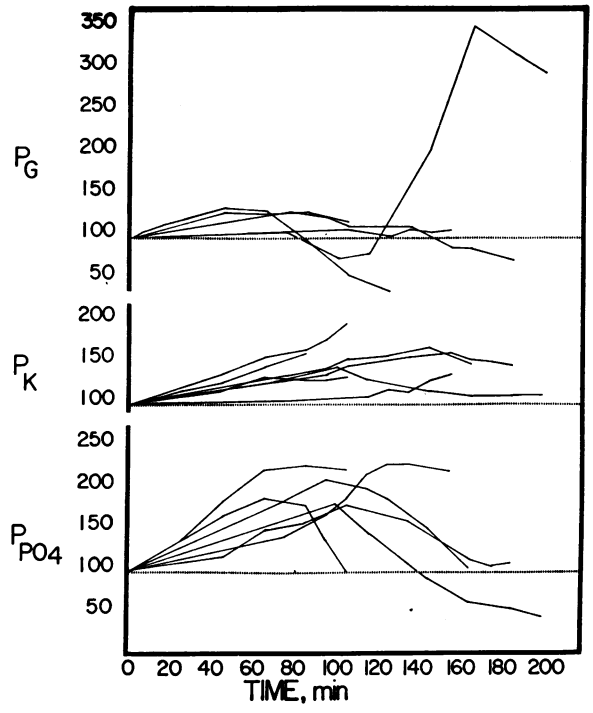

FIGURE 1 Effect of salicylate on plasma concentrations of glucose, potassium, and phosphate. Salicylate was administered by continuous intravenous infusion beginning at zero time. The data are presented relative to values of the control periods. The means of two consecutive control periods were set equal to 100.

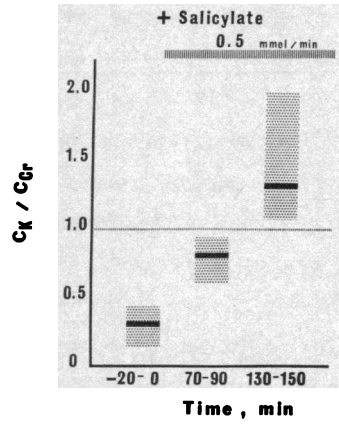

FIGURE 2 Effect of salicylate on potassium clearance ratios. The shadowed areas represent the range for data of eight experiments and the black bars represent the means. Intravenous salicylate infusion was begun at zero time. Potassium clearance increased progressively with time and exceeded the creatinine clearance after 130 min of infusion.

The effects of salicylate on plasma glucose concentration and glucose tubular reabsorption are presented in Table V. In four of six experiments glycosuria occurred in the absence of hyperglycemia and only on the side infused with salicylate.

Effect of salicylate on adenine nucleotides of the kidney. The concentrations of ATP, ADP, and AMP in cortex and medulla are presented in Table VI. Control data refer to concentrations reported elsewhere for a series of 10 normal dogs subjected to experimental conditions similar to those used in the present study (15). In both studies the techniques of removal and freezing of the tissues were identical. In the cortex, the mean content of ATP in salicylate treated dogs was 0.37±0.06 SEM μmol/g wet weight, against 0.76±0.05 μmol/g wet weight in the controls. AMP content was 0.77±0.05 μmol/g wet weight in the salicylate-treated dogs and 0.41±0.04 μmol/g wet weight in normal dogs. These differences were statistically significant by the double-tailed *t* test. There was no statistically significant difference in ADP content in the cortex between control and experimental dogs.

Control data for the medulla are from unpublished studies of one of the authors in six normal dogs (R. H. Kessler). The mean concentration of ATP was essentially equal in both series, but AMP and ADP were significantly higher in the salicylate-treated dogs than in the control dogs.

TABLE III

Infusion of Salicylate into the Left Renal Artery

Experiment no.	Time	V		C_{Cr}		C_{PO_4}		C_{Na}		C_K	
		Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
		ml/min		*ml/min*		*ml/min*		*ml/min*		*ml/min*	
29	−20–10	1.9	1.8	47	47	14.7	15.1	2.78	2.59	14.5	14.2
	−10–0	1.8	1.6	48	46	14.3	13.8	2.70	2.38	13.2	12.4
		+Salicylate 0.32 mmol/min									
	0–10	1.7	3.5	46	46	13.9	17.5	2.59	4.50	12.7	19.3
	10–20	1.3	3.9	45	43	12.7	18.8	2.00	4.70	11.7	22.8
	20–30	0.7	3.0	41	42	11.5	19.7	1.08	3.41	9.6	21.8
	30–40	0.6	2.9	45	40	14.9	21.6	0.94	3.27	10.9	22.0
32	−20–10	1.3	1.6	26	26	9.1	9.7	1.65	1.97	6.2	6.8
	−10–0	1.3	1.5	25	25	9.1	9.4	1.64	1.92	5.9	6.4
		+Salicylate 0.43 mmol/min									
	0–10	1.5	3.5	25	26	9.0	12.8	2.03	3.93	6.4	11.3
	10–20	1.2	4.1	24	21	9.7	13.9	1.62	4.54	6.4	12.8
	20–30	1.1	3.7	24	20	11.5	14.2	1.43	4.06	8.9	13.7
	30–40	1.2	4.1	23	20	12.3	15.7	1.59	4.46	11.0	15.1
33	−20–30	0.8	0.7	39	43	9.7	10.4	1.16	1.16	6.1	5.3
	−10–0	0.6	0.5	37	40	8.0	9.2	0.91	0.92	3.1	4.1
		+Salicylate 0.5 mmol/min									
	0–10	0.3	1.5	36	51	8.1	15.5	0.55	2.26	3.6	10.0
	10–20	0.4	3.1	40	40	10.6	19.1	0.64	2.83	4.6	15.2
	20–30	0.4	3.9	34	39	11.9	23.9	0.70	4.68	4.6	15.2
	30–40	0.7	5.1	41	40	17.8	28.0	1.03	5.90	9.8	24.5
34	−20–10	0.9	0.8	53	54	8.6	8.1	1.59	1.47	7.4	6.5
	10–0	0.9	0.9	50	52	7.4	7.0	1.69	1.70	7.6	6.6
		+Salicylate 0.5 mmol/min									
	0–10	1.2	1.8	55	51	8.9	13.1	2.00	2.85	11.2	17.2
	10–20	1.6	2.4	49	50	12.1	16.3	2.57	3.52	17.3	23.8
	20–30	1.9	2.8	52	53	17.1	21.0	3.01	4.01	21.7	28.8
	30–40	1.9	3.6	47	45	19.5	24.2	3.05	5.06	24.4	31.6
35	−20–10	2.1	2.5	45	45	3.7	5.2	3.27	3.63	12.2	12.7
	−10–0	1.9	2.3	43	44	3.4	5.0	2.99	3.36	10.5	11.2
		+Salicylate 0.5 mmol/min									
	0–10	2.3	3.7	46	44	4.9	12.8	3.47	5.04	11.1	17.4
	10–20	2.2	4.6	45	44	4.1	17.9	3.28	5.71	11.9	21.9
	20–30	2.0	4.5	46	42	3.2	16.6	3.08	5.33	13.8	26.4
	30–40	1.0	4.4	48	39	2.7	12.5	1.53	4.84	13.3	26.1
36	−20–10	3.2	4.3	48	51	11.7	14.5	3.65	4.69	13.2	15.2
	−10–0	3.2	4.1	51	53	12.1	14.6	3.61	4.61	13.9	15.8
		+Salicylate 0.5 mmol/min									
	0–10	3.1	4.6	47	50	12.4	16.2	3.53	5.24	13.5	17.9
	10–20	2.5	4.9	47	49	11.5	18.2	2.93	5.51	12.0	19.1
	20–30	2.7	6.3	48	53	13.2	22.0	2.78	6.18	14.5	23.9
	30–40	3.4	7.2	46	51	16.5	28.1	2.91	6.16	19.1	28.1
	40–50	3.8	8.0	45	45	18.6	29.3	3.08	6.94	23.8	32.6
	50–60	4.3	8.3	43	45	20.7	31.0	3.70	7.72	30.0	36.4
37	−20–10	1.2	1.3	36	20	4.6	4.9	1.65	2.06	5.4	6.6
	−10–0	2.4	2.7	41	39	7.0	9.6	3.57	4.50	11.2	13.9
		+Salicylate 0.5 mmol/min									
	0–10	2.7	3.6	43	42	9.9	10.9	4.44	5.73	12.1	15.7
	10–20	2.7	4.6	43	41	11.1	16.0	4.82	7.65	13.6	21.7
	20–30	2.5	3.9	43	40	12.7	17.5	4.46	6.45	16.7	24.7
	30–40	2.6	4.9	45	44	15.6	28.9	4.46	8.12	17.7	30.6
	40–50	3.1	6.5	47	45	—	—	4.90	9.02	25.9	39.7
	50–60	3.5	9.4	48	42	—	—	5.43	9.98	30.6	35.8
Mean ±SEM	Controls	1.7±0.3	1.9±0.5	42±3	43±4	8.7±1.4	9.8±1.4	2.35±0.35	2.64±0.47	9.3±1.4	9.8±1.5
	Last period	1.9±0.6	5.4±0.9	42±3	39±3	14.8±2.3	23.1±2.6	2.47±0.63	5.89±0.86	18.6±3.6	27.4±2.9

Data of right and left kidney in seven experiments. After two control periods salicylate was injected into the left renal artery.

TABLE IV

Infusion of Sodium Chloride into the Left Renal Artery

Experiment no.	Time	V		C_{Cr}		C_{PO_4}		C_{Na}		C_K	
		Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
	min	*ml/min*		*ml/min*		*ml/min*		*ml/min*		*ml/min*	
1	−20–10	1.12	1.44	42.6	31.5	10.3	7.9	1.56	1.67	4.48	3.20
	−10–0	1.21	1.60	42.7	32.0	10.9	8.4	1.67	1.83	5.41	3.90
		+Sodium chloride, 0.5 mmol/min									
	0–10	1.48	2.18	44.4	34.3	11.7	9.5	1.91	2.35	6.66	5.51
	10–20	1.66	2.43	45.3	34.5	12.0	9.7	2.13	2.60	8.20	6.85
	20–30	1.68	2.23	44.7	34.1	11.7	9.8	2.04	2.40	8.75	7.21
	30–40	1.72	2.28	46.1	34.7	12.1	9.9	2.10	2.48	8.75	7.24
2	−20–10	2.38	3.64	34.8	28.1	18.4	15.8	1.32	2.72	20.4	15.2
	−10–0	2.18	3.54	30.7	35.1	12.4	15.8	1.22	2.76	14.1	15.3
		+Sodium chloride, 0.5 mmol/min									
	0–10	2.0	3.18	27.1	31.3	12.5	16.1	1.25	2.61	12.6	15.3
	10–20	1.7	2.96	27.5	33.0	12.8	16.5	1.16	2.54	1.05	14.0
	20–30	1.55	2.85	29.4	34.1	13.6	17.8	1.27	2.75	11.2	15.3
	30–40	1.55	2.70	27.0	31.9	13.2	17.4	1.19	2.61	10.5	14.3
3	−20–30	3.05	3.03	38.5	37.9	9.9	8.1	2.64	2.40	13.2	11.6
	−10–0	3.33	3.32	37.5	37.8	9.7	9.1	2.61	2.53	11.8	12.0
		+Sodium chloride, 0.5 mmol/min									
	0–10	3.50	3.72	38.6	38.2	10.2	10.3	2.71	2.86	12.6	12.6
	10–20	3.60	4.00	39.0	38.4	11.0	9.7	2.72	3.14	12.1	12.8
	20–30	3.73	4.08	38.1	38.3	11.0	10.3	2.38	3.18	10.7	13.8
	30–40	3.57	4.07	40.2	39.7	12.1	11.7	2.52	2.98	13.3	14.0
	40–50	3.65	4.10	39.7	39.8	12.7	13.7	2.54	2.93	12.7	14.9
	50–60	3.15	3.65	37.9	33.9	14.4	13.7	2.28	2.66	13.2	18.9
4	−20–10	2.3	3.4	37.2	40.8	12.8	14.2	1.42	2.38	8.1	11.5
	−10–0	2.7	3.3	37.3	39.0	15.0	13.8	1.56	2.18	9.3	12.1
		+Sodium chloride, 0.5 mmol/min									
	0–10	2.0	3.0	34.8	38.3	10.5	12.2	1.07	1.91	8.3	12.1
	10–20	2.1	2.4	36.8	34.8	11.2	11.0	1.12	1.54	8.9	10.6
	20–30	2.0	2.6	36.1	39.0	9.7	11.2	1.08	1.61	8.1	10.8
	30–40	2.0	2.8	34.3	35.7	9.2	10.2	1.07	1.81	7.9	11.3
Mean ±SEM	Control	2.3±0.4	2.9±0.5	37.7±2	35±3	12.4±1.3	11.6±1.9	1.75±0.3	2.30±0.2	10.9±2.6	10.6±2.5
	Last period	2.1±0.4	2.9±0.3	36.3±4	34±1	12.2±1.1	12.8±1.8	1.7±0.3	2.39±0.2	10.1±1.2	12.9±2.5

Data of right and left kidney in four experiments. After two control periods sodium chloride 0.5 mmol/min was infused into the left renal artery.

Concentration of salicylate in plasma and kidney tissue. Salicylate concentrations in plasma and kidney tissues are presented in Table VII. Tissue concentrations measured in six experiments of the intraarterial infusion type, are presented in the table. Systemic plasma salicylate concentration varied between 0.33 and 0.52 mg/ml. The salicylate concentration in the injected renal artery was calculated to be about 1 mg/ml assuming a renal plasma flow of 3 times GFR. The concentration of salicylate was measured in cortex and outer and inner medulla of the left kidney. In every experiment there was a gradient in the concentration of salicylate from cortex to inner medulla that increased by a factor of 1.5 to 3. Chloride concentrations measured in these same tissue samples showed similar concentration gradients. These differences in concentration between cortex, outer medulla, and inner medulla were statistically significant. In experiments 27 and 28 of the systemic infusion variety (not presented in this table) the plasma salicylate concentration varied between 0.74 and 1.08 mg/ml, respectively, during the last experimental period. Salicylate clearances measured during these same periods varied between 8.4 and 11.2 ml/min.

DISCUSSION

We examined the acute effects of salicylate by injecting quantities sufficient to produce concentrations of salicylate of about 1 mg/ml of plasma. These levels are comparable to concentrations reported in the literature in cases of severe human intoxication (5, 9, 24).

Salicylate exerted a marked and prompt diuretic action. The diuresis was associated with an enhanced excretion of sodium, potassium, chloride, phosphate

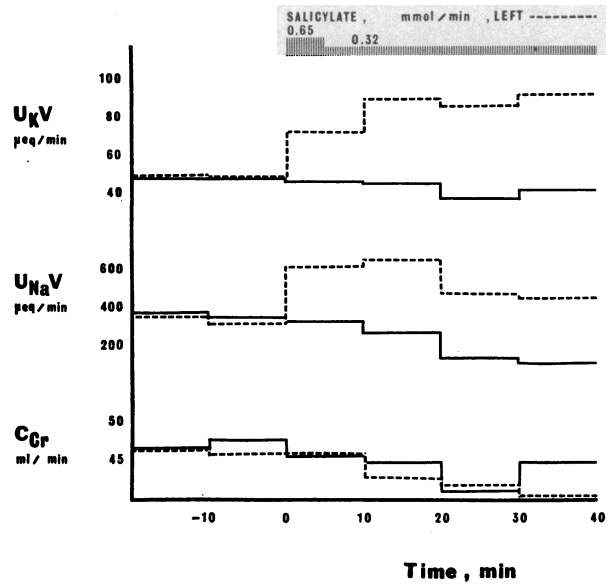

FIGURE 3 Experiment 29. Salicylate was administered by continuous infusion into the left renal artery. The graph demonstrates the effect of salicylate on creatinine clearance, sodium excretion, and potassium excretion of left and right kidneys.

and, occasionally, glucose. Because the sodium salt of salicylate was used in this study, it could be alleged that the natriuresis was a reflection of excretion of this salt. This was not the case because the excretion rate of the salicylate anion was only 5–15% of the rate of sodium excretion on a stoichiometric basis. The bulk of sodium excretion was accompanied by chloride and not salicylate as the associated anion; the nonreabsorbed fraction of chloride almost equalled that of sodium. From our results it appears possible that an early diuresis may contribute to the dehydration that accompanies salicylate poisoning. Oliguria, frequently described, is more likely a late effect secondary to extrarenal water loss with concentration of the extracellular volume and reduction of filtration rate.

Potassium excretion was enhanced, occasionally exceeding the potassium filtered load. Increased potassium excretion, frequently observed in salicylate poisoning, has been attributed to respiratory alkalosis secondary to hyperventilation (8, 9). However, our results indicate that the kaliuretic effect is a direct one and at least partially independent of alkalosis.[1]

When salicylate was administered directly into one renal artery, kaliuresis was immediate, marked, and predominantly or exclusively from the injected kidney. Furthermore, when salicylate was injected intravenously and hyperventilation occurred, the administration of 5% CO_2 in the inspired gas did not prevent enhancement of potassium excretion.

Salicylate also caused a prompt increase in potassium and phosphate concentration in plasma followed later by a phase of reduced concentrations of these ions. The early increase may have been due to an inhibitory effect on the transport system which maintains the high intracellular concentrations of these ions. This presumption is supported by the studies of Manchester et al. in rat diaphragm in which salicylate caused a loss of intracellular potassium (25). The explanation offered by these authors was that salicylate poisons a potassium pump in cell membranes diminishing potassium influx. However, Hicklin has demonstrated that decreased intracellular potassium was the result of enhanced efflux of this cation (26). Whatever the mechanism, the only possible source of potassium and phosphate to account for the early hyperkalemia and hyperphosphatemia is the intracellular compartment.

In some experiments, a fall in plasma concentrations of potassium and phosphate was noted about 30–60 min after administration of salicylate. This later change prompted us to prolong the duration of observation in some experiments. Eventually plasma potassium and phosphate concentrations fell below control period levels. Such hypokalemia and hypophosphatemia may result from a urinary loss which eventually exceeds the rate of efflux from cells.

Glycosuria, when observed in salicylate poisoning, has been interpreted to be secondary to hyperglycemia (27). From our data salicylate appears to block tubular glucose reabsorption directly. Glycosuria occurred unilaterally when salicylate was administered into one renal artery. Furthermore, glucose appeared in the urine at filtered loads far below the minimum reabsorptive capacity of the dog kidney.

Hyperglycemia is known to occur in salicylate toxicity (6, 28), and, in fact, was noted in all experiments after intravenous infusion of salicylate. In some cases the effect was biphasic; the early hyperglycemia was followed by a late decline. The explanation for the initial hyperglycemia is not clear. However, it has

[1] Because salicylate is incompletely reabsorbed, it could be expected to increase the electronegativity within the lumen of the distal tubule and, in this manner, enhance the excretion of potassium in a fashion similar to sulfate or ferrocyanate. Were this the sole explanation for the kaliuresis associated with the administration of salicylate, there should be at most a 1 to 1 stoichiometric relationship in the rates of excretion of potassium and salicylate. In experiments 25 and 27 this relationship was examined; the rates of salicylate excretion equalled 73 and 61 μmole/min, while potassium excretion in the same collection periods had increased to 164 and 160 μmole/min from controls of 5 and 14 μmole/min, respectively. We conclude, therefore, that the presence of unreabsorbed salicylate anion could account for only a fraction of the increment in the rate of potassium excretion.

TABLE V

Infusion of Salicylate into the Left Renal Artery Effect on Glucose Reabsorption

Experiment no.	Time	P_G	U_GV Right	U_GV Left	G Reabsorbed Right	G Reabsorbed Left
	min	*mg/ml*	*mg/min*		*mg/min*	
32	−20–10	0.88	0	0	23	23
	−10–0	0.92	0	0	23	23
	+Salicylate 0.43 mmol/min					
	0–10	0.92	0	0.64	23	23
	10–20	0.93	0	2.17	22	18
	20–30	0.94	0	3.33	23	16
	30–40	0.93	0	3.97	21	15
33	−20–10	1.09	0	0	42	46
	−10–0	1.12	0	0	41	45
	+Salicylate 0.5 mmol/min					
	0–10	1.14	0	0	41	58
	10–20	1.16	0	0	46	46
	20–30	1.19	0	0	41	47
	30–40	1.23	0	0	50	49
34	−20–10	1.11	0	0	58	60
	−10–0	1.08	0	0	54	57
	+Salicylate 0.5 mmol/min					
	0–10	1.15	0	0	63	58
	10–20	1.15	0	0	56	57
	20–30	1.17	0	0	61	61
	30–40	1.26	0	0	59	56
35	−20–10	0.93	0	0	42	42
	−10–0	0.98	0	0	42	43
	+Salicylate 0.5 mmol/min					
	0–10	1.05	0	0	48	47
	10–20	1.02	0	1.99	46	43
	20–30	1.08	0	4.01	49	42
	30–40	1.10	0	16.6	52	26
36	−20–10	0.98	0	0	43	46
	−10–0	0.88	0	0	44	47
	+Salicylate 0.5 mmol/min					
	0–10	0.83	0	0	39	41
	10–20	0.74	0	0	35	36
	20–30	0.83	0	8.83	40	35
	30–40	0.83	0	11.9	38	30
	40–50	0.86	0	16.2	39	22
	50–60	0.88	0	23.0	38	17
37	−20–10	0.92	0	0	33	19
	−10–0	0.92	0	0	38	36
	+Salicylate 0.5 mmol/min					
	0–10	0.96	0	0	41	41
	10–20	0.93	0	0	40	38
	20–30	1.02	0	1.04	44	39
	30–40	1.01	0	1.30	46	43
	40–50	1.04	0	7.0	49	39
	50–60	1.03	0	18.1	49	25
Mean ±SEM						
Controls		0.84 ±0.14	0	0	35	35
Last period		0.92 ±0.16	0	8.81 ±3.79	38 ±8	27 ±7

Data of plasma glucose concentration, urinary glucose excretion and glucose reabsorption in six experiments. After two control periods salicylate was injected into the left renal artery.

TABLE VI

Adenine Nucleotides Content in Kidney Tissue

Experiment no.	Cortex AMP	Cortex ADP	Cortex ATP	Medulla AMP	Medulla ADP	Medulla ATP
26	0.73	0.88	0.39	0.19	0.31	0.41
27	0.92	0.69	0.22	0.17	0.32	0.32
28	0.73	0.84	0.37	0.35	0.41	0.34
33	0.69	0.68	0.52	0.13	0.13	0.45
Mean	0.77	0.77	0.37	0.21	0.29	0.38
±SEM	±0.05	±0.05	±0.06	±0.05	±0.05	±0.03
Controls	0.41	0.86	0.76	0.09	0.18	0.41
±SEM	±0.04	±0.03	±0.05	±0.01	±0.02	±0.02
n = 10			n = 6			
2P	0.001	0.10	0.005	0.01	0.05	0.2

Means and standard errors of the mean for adenine nucleotide content in the left kidney cortex and medulla after administration of salicylate. Values shown are in micromoles per gram of wet weight. Control values for cortex are from previous studies (15). Control values for medulla are from unpublished data (R. H. Kessler).

been suggested that salicylate increases the rate of glycogenolysis (28).

We can only speculate on the mechanism by which salicylate inhibits sodium, potassium, phosphate, and glucose transport. Ramsay and Elliott have shown that several ortho-substituted congeners of benzoic acid, including the *o*-hydroxy substitution (salicylate), enhance urinary excretion of electrolytes although the *o*-acetoxy substitution (aspirin) did not (12). These results are consistent with the suggestion that salicylate has a direct inhibitory action on the tubule. Perhaps the natriuretic effect of salicylate is related to its action as a metabolic inhibitor. There are several sites in the metabolic pathways where salicylate could theoretically interfere with provision of energy. It is an uncoupler of oxidative phosphorylation in vitro (29). In the uncoupled kidney one would predict a decreased concentration of ATP and an increased concentration of AMP in cortical tissue. These changes were, in fact, observed in our experiments. Our interpretation that this fall results from uncoupling of cortical metabolism, is reinforced by the observation that medullary ATP concentrations was not altered by salicylate despite their increased concentration in the medulla relative to the cortex. We suggest that in a tissue such as the inner medulla that is primarily glycolytic (30), it is unlikely that an uncoupler of oxidative phosphorylation would alter the content of ATP.

Salicylate also is known to inhibit, in vitro, several enzymes of the Krebs cycle such as ketoglutarate and succinate dehydrogenases (31) as well as malic and isocitric dehydrogenases (32). These inhibitory effects of salicylate have been reported with concentrations similar to those attained in the plasma of dogs used in this study. These actions of salicylate could also

TABLE VII

Salicylate and Chloride Concentration in Kidney Tissue

Experiment no.	Plasma salicylate	Salicylate			Chloride		
		Cortex	Outer medulla	Inner medulla	Cortex	Outer medulla	Inner medulla
	mg/ml		*mg/g*			*μeq/g*	
29	0.34	0.12	0.24	0.39	42.0	95.0	141
33	0.35	0.20	0.28	0.35	51.3	109.0	104
34	0.33	0.19	0.31	0.45	48.3	83.8	99.8
35	0.38	0.17	0.38	0.40	37.5	83.0	88.3
36	0.49	0.31	0.40	0.45	43.6	73.0	79.5
37	0.52	0.24	0.31	0.37	54.0	84.0	107
Mean ±SEM	0.40±0.02	0.20±0.03	0.32±0.02	0.40±0.01	46.1±2.5	88.0±5.1	103.3±8.6
			$P < 0.001$	$P < 0.025$		$P < 0.01$	$P < 0.10$
			$P < 0.0025$			$P < 0.0025$	

Means and standard errors of the mean for plasma salicylate and for salicylate and chloride concentration in the kidney cortex, outer medulla, and inner medulla after 40–60 min of infusion of salicylate into the renal artery. Amount injected was between 0.32 and 0.5 mmol/min. Dogs were undergoing saline diuresis.

result in a reduction of energy for active transport. Our observations are consistent with the hypothesis that oxidative phosphorylation provides a part of the energy for active ion transport in the kidney.

The renal lesions of analgesic nephropathy are chiefly localized in the papilla and deep medulla. Such distribution of lesions could be explained if the offending drugs were concentrated in the kidney tissue. Bluemle and Goldberg reported that they could find no difference in salicylate concentration between cortex and medulla in the dog kidney (33) in contradiction with our results. Salicylate in our studies was concentrated in the medulla by a factor of 1.5–3 with respect to the cortex. This concentration gradient of salicylate was almost identical with that of chloride. Because during saline diuresis chloride is concentrated in the medulla secondary to the countercurrent system, the similarity of chloride and salicylate gradients suggests that salicylate may be concentrated by the same mechanism.

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REFERENCES

1. Smith, M. J. H., and P. K. Smith. 1966. The Salicylates. A Critical Bibliographic Review. John Wiley & Sons, Inc., New York. 267.
2. Cumming, G., D. C. Dukes, and G. Widdowson. 1964. Alkaline diuresis in treatment of aspirin poisoning. *Br. Med. J.* **2**: 1033.
3. Robin, E. D., R. P. Davis, and S. B. Rees. 1959. Salicylate intoxication with special reference to the development of hypokalemia. *Am. J. Med.* **26**: 869.
4. Charnock, J. S., L. J. Opit, and B. S. Hetzel. 1961. Electrolyte distribution in rats following salicylate. *Metab. (Clin. Exp.).* **10**: 874.
5. Morgan, A. G., and A. Polak. 1969. Acetazolamide and sodium bicarbonate in treatment of salicylate poisoning in adults. *Br. Med. J.* **1**: 16.
6. Segar, W. E., and M. A. Holliday. 1958. Physiologic abnormalities of salicylate intoxication. *N. Engl. J. Med.* **259**: 1191.
7. Limbeck, G. A., R. H. A. Ruvalcaba, E. Samols, and V. C. Kelley. 1965. Salicylates and hypoglycemia. *Am. J. Dis. Child.* **109**: 165.
8. Winters, R. W. 1959. Salicylate intoxication in infants and children. *Pediatr. Clin. North Am.* **6**: 281.
9. Ferguson, R. K., and A. R. Boutros. 1970. Death following self-poisoning with aspirin. *J. Am. Med. Assoc.* **213**: 1186.
10. Hetzel, B. S., J. S. Charnock, and H. Lander. 1959. Metabolic effects of salicylate in man. *Metab. (Clin. Exp.).* **8**: 205.
11. Blane, G. F., and K. A. Munday. 1961. Effects of salicylate administration on reputed indices of adrenal cortical activity in the rat. *J. Pharm. Pharmacol.* **13**: 595.
12. Ramsay, A. G., and H. C. Elliott. 1967. Effect of acetylsalicylic acid on ionic reabsorption in the renal tubule. *Am. J. Physiol.* **213**: 323.
13. Smith, M. J. H., and P. K. Smith. 1966. The Salicylates. A Critical Bibliographic Review. John Wiley & Sons, Inc., New York. 273.
14. Franklin, S. S., J. T. August, S. B. Rees, E. D. Robin, and J. P. Merril. 1959. The effect of salicylate on renal

tubular transport of PAH, phosphate and potassium. *Res. Proc.* **7**: 276.
15. Kessler, R. H. 1970. Effects of ischemia on the concentration of adenine nucleotides in the kidney of anesthetized dogs. *Proc. Soc. Exp. Biol. Med.* **134**: 1091.
16. Urbaitis, B. K., and R. H. Kessler. 1969. Concentration of adenine nucleotide compounds in renal cortex and medulla. *Nephron.* **6**: 217.
17. Cohen, W. F., and C. E. Carter. 1950. The separation of adenosine polyphosphates by ion exchange and paper chromatography. *J. Am. Chem. Soc.* **72**: 4273.
18. Kessler, R. H. 1960. Acute effects of brief ureteral stasis on urinary and renal papillary chloride concentration. *Am. J. Physiol.* **199**: 1215.
19. Smith, H. W. 1956. Principles of Renal Physiology. Oxford University Press, Inc., New York. 213.
20. Smith, H. W., N. Finkelstein, L. Aliminosa, B. Crawford, and M. Graber. 1945. The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. *J. Clin. Invest.* **24**: 388.
21. Chen, P. S., Jr., T. Y. Toribara, and H. Warner. 1956. Microdetermination of phosphorus. *Anal. Chem.* **28**: 1756.
22. Brodie, V. V., S. Udenfriend, and A. F. Coburn. 1943. The determination of salicylic acid in plasma. *J. Pharmacol. Exp. Ther.* **80**: 114.
23. Washko, M. E., and E. W. Rice. 1961. Determination of glucose by an improved enzymatic procedure. *Clin. Chem.* **7**: 542.
24. Brown, S. S., J. C. Cameron, and H. Mathew. 1967. Plasma salicylate levels in acute poisoning in adults. *Br. Med. J.* **2**: 738.
25. Manchester, K. L., P. J. Randle, and G. H. Smith. 1958. Some effects of sodium salicylate on muscle metabolism. *Br. Med. J.* **1**: 1028.
26. Hicklin, J. A. 1959. Salicylate and potassium fluxes of rat diaphragm. *Nature (Lond.)*. **184**: 2029.
27. Smith, M. J. H. 1966. Metabolic effects of salicylates. *In* The Salicylates. M. J. H. Smith and P. K. Smith, editors. John Wiley & Sons, Inc., New York. 49.
28. Smith, M. J. H. 1955. The effects of sodium salicylate on blood glucose in the rat. *Br. J. Pharmacol.* **10**: 110.
29. Thompkins, L., and K. H. Lee. 1969. Studies on the mechanism of action of salicylates. IV. Effect of salicylates on oxidative phosphorylation. *J. Pharmacol. Sci.* **58**: 102.
30. Bernanke, D., and F. H. Epstein. 1965. Metabolism of the renal medulla. *Am. J. Physiol.* **208**: 541.
31. Kaplan, E. H., J. Kennedy, and J. Davis. 1954. Effects of salicylate and other benzoates on oxidative enzymes of the tricarboxylic acid cycle in rat tissue homogenates. *Arch. Biochem.* **51**: 47.
32. Hines, W. J. W., and M. J. H. Smith. 1964. Inhibition of dehydrogenases by salicylate. *Nature (Lond.)*. **201**: 192.
33. Bluemle, L. W., and M. Goldberg. 1968. Renal accumulation of salicylate and phenacetin: possible mechanisms in the nephropathy of analgesic abuse. *J. Clin. Invest.* **47**: 2507.