The Effect of Methyl Acetylsalicylate on Renal Tubular Ionic Reabsorption[1] (34203)

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Previous work demonstrated that plasma concentrations of acetylsalicylic acid of 2–4 mg/100 ml were associated with a 50% reduction in the excretory rates of Na^+, Cl^-, Ca^{2+}, Mg^{2+}, and HCO_3^- in the dog (1). There was no change in acid–base status, GFR and plasma ionic concentrations. It was concluded that ASA had a specific effect on the renal tubule by which the reabsorption of these various ions was increased. The effect on tubular reabsorption was specific for ASA. Three other congeners of benzoic acid including *O* ethoxy benzoate, *O*-acetyl benzoate, and sodium salicylate did not increase tubular reabsorption. It was suggested that the acetoxy substitution on benzoic acid gave the biologic action on the renal tubule.

The present study was undertaken to further investigate the chemical specificity of the phenomenon. The effect of methyl acetylsalicylate (methyl ASA) on tubular reabsorption was studied by clearance experiments in dogs. It was found that this compound had an action quite similar to that of ASA and produced a significant reduction in the excretion of Na^+, Cl^-, HCO_3^-, Ca^{2+} and Mg^{2+} without reduction in ionic filtered loads. This indicated that methyl ASA augments renal tubular reabsorption of these ions.

Methods. Clearance experiments were performed in 6 mongrel dogs which were anesthetized with pentobarbital sodium 30 mg/kg. The dogs weighed from 12 to 18 kg. Both ureters were cannulated with PE no. 190 tubing, either through flank incisions or through a midline abdominal incision. Urine samples were collected under mineral oil from both kidneys in the same graduated cylinder. The animals were infused intravenously at a rate of 5.3 ml/min with NaCl, 0.4%; mannitol, 2%; and inulin, 0.5%. A prime of 1 g of inulin was administered to each animal at the commencement of infusion. The femoral artery was cannulated for blood sampling and blood pressure measurement with an Hg manometer. Two dogs were infused with 0.06% PAH after a prime injection of 120 mg. The left renal vein of these animals was cannulated with PE no. 20 tubing through the ovarian vein. After a suitable period during which urine flow stabilized, four 10-min control urine collections were made. An arterial blood sample was taken anaerobically in an oiled heparinized syringe at the midpoint of each collection. Renal venous blood was also sampled at the midpoint of each period. Following the fourth collection period, the dogs were given an intravenous prime of 120 mg of methyl acetylsalicylate. An appropriate amount of the compound was added to the infusate so that these animals received it at the rate of 1 mg/min. After a 30-min equilibration period, six or seven 10 min urine collections with midpoint blood samples were obtained. At the conclusion of experiments, the left kidney was removed for tissue analysis of methyl acetylsalicylate.

Inulin in plasma and urine was determined by the method of Schreiner (2), PAH by the method of Smith (3), chloride by the amperometric titration method of Cotlove (4), Na^+ and K^+ by flame photometry, Ca^{2+} by the method of Appleton *et al.* (5), Mg^{2+} by the method of Schacter (6), and osmolality with an Advanced Instruments osmometer. The pH was determined with a Radiometer pH meter and total CO_2 by the manometric method of Van Slyke and Neill (7). The HCO_3^- in urine and plasma was calculated from the Henderson-Hasselbalch

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TABLE I. Effect of Methyl Acetylsalicylate on Blood Pressure (BP), Renal Plasma Flow (RPF), Inulin Clearance (C^{In}), Protein Concentration of Efferent Arteriole Plasma, Osmolar Clearance (C^{Osm}) and Reabsorption of Solute-Free Water ($T_{C_{H_2O}}$).

Time (min)	BP	C^{PAH} (ml/min)	C^{In} (ml/min)	$\frac{C^{In}}{C^{PAH}}$	Femoral artery plasma protein (g/100 ml)	Efferent arteriole plasma protein (g/100 ml)[a]	Urine flow (ml/min)	C^{Osm} (ml/min)	$T_{C_{H_2O}}$ (ml/min)
90–100	110	108	51.6	.48	4.4	8.5	1.36	2.53	1.17
100–110	105	116	40.0	.34	4.3	6.5	1.34	2.57	1.23
110–120	105	136	49.6	.36	4.2	6.6	1.46	2.90	1.44
120–130	110	124	56.1	.45	4.2	7.3	1.52	3.01	1.49
131	Methyl acetylsalicylate prime 120 mg; start infusion at 1 mg/min								
131–160	Equilibration								
160–170	110	170	61.9	.36	4.1	6.4	1.50	3.31	1.81
170–180	110	135	40.8	.30	4.2	6.00	1.20	2.54	1.34
180–190	110	107	52.1	.49	4.1	8.00	1.10	2.36	1.26
190–200	110	108	52.6	.49	4.0	7.8	1.10	2.38	1.28
200–210	110	119	73.5	.62	3.6	9.5	1.10	2.24	1.14
210–220	100	112	60.4	.54	3.6	7.8	0.92	1.93	1.00
220–230	105	142	63.4	.45	3.5	6.4	1.20	2.55	1.35
Mean control	108	121	49.3	.41	4.3	7.2	1.42	2.75	1.33
Mean exptl.	108	128	58.0	.46	3.9	7.4	1.16	2.47	1.31
Δ	0	+7	+8.7	+.05	−0.4	+0.2	−0.26	−0.28	−0.02

[a] Efferent arteriole plasma protein = femoral artery plasma protein/$1-(C^{In}/C^{PAH})$.

TABLE II. Effect of Methyl Acetylsalicylate on Ionic Excretion.

Time (min)	Filtered load (μmoles/min)			Urinary excretion (μmoles/min)						Arterial pCO_2
	Na^+	Cl^-	HCO_3^-	Na^+	Cl^-	Ca^{2+}	Mg^{2+}	K^+	HCO_3^-	
90–100	6520	5532	1238	99	71	2.12	0.61	42.8	17.3	43
100–110	5320	4225	1024	91	74	1.81	0.72	52.9	17.2	38
110–120	6267	5317	1200	103	90	2.44	0.64	72.3	22.0	36
120–130	6715	6173	1346	122	103	2.55	0.33	71.4	23.0	35
131	Methyl acetylsalicylate prime 120 mg; start infusion at 1 mg/min									
131–160	Equilibration									
160–170	7703	6422	1374	84	63	1.35	0.26	51.0	10.4	32
170–180	4961	4181	857	49	48	1.42	0.16	41.4	5.4	30
180–190	6187	5290	1155	47	47	1.42	0.20	45.1	4.4	34
190–200	6696	5578	1057	33	37	1.10	0.41	38.5	2.2	27
200–210	9217	7903	1566	33	46	1.30	—	37.4	2.2	29
210–220	7632	6342	1105	12	20	0.86	0.20	29.4	1.0	24
220–230	7348	6644	1439	10	13	0.89	0.22	35.4	2.3	28
Mean control	6206	5312	1202	106	85	2.23	0.57	60.0	19.9	38
Mean exptl.	7106	6051	1222	38	39	1.19	0.24	39.5	4.0	29
Δ	+900	+739	+20	−68	−46	−1.04	−0.33	−20.5	−15.9	−9

equation. A CO_2 solubility coefficient of .0301 was used in plasma; and in urine a value of .0309 was employed. The clearance of inulin (C^{In}) was regarded as the glomerular filtration rate (GFR). The PAH clearance (C^{PAH}) was taken as the value for renal cortical plasma flow. The PAH extraction (E^{PAH}) was calculated from $E^{PAH} = (A—R)/A$ where A is arterial PAH concentration and R is renal venous concentration of PAH. Renal plasma flow (RPF) was calculated from the ratio C^{PAH}/E^{PAH}. Reabsorption of solute-free water ($TC\ H_2O$) was calculated from osmolar clearance (C^{Osm}) — rate of urine flow (V). Filtered loads of univalent ions were calculated as the product of plasma concentration, C^{In} and the appropriate Donnan factor (0.95 for cations and 1.05 for anions).

Methyl acetylsalicylate was measured in tissue by a spectrophotometric method. 200 to 300 mg of tissue were ground in 5 ml of 20% ethyl alcohol to make an emulsion. The emulsion was extracted with 10 ml of ethylene dichloride for 45 min on a mechanical shaker. The mixture was then centrifuged for 10–15 min at 3500 rpm. The absorbence of the ethylene dichloride extract of tissue was determined in a Beckman DU spectrophotometer at 276 and 310 mμ. Methyl acetylsalicylate gave maximum absorbence at 276 mμ. The maximal absorbence for methyl salicylate was found to occur at 310 mμ. Slight nonspecific absorbence was found at both wavelengths. A small amount of absorbence at 276 mμ was due to methyl salicylate. This value was added to the blank and the sum was subtracted from the initial reading. Concentrations of both methyl acetylsalicylate and methyl salicylate were then determined from a standard curve.

Results. A typical clearance experiment in one animal is shown in Tables I and II. The first four periods were controls. Subsequently the animal was primed with 120 mg of methyl acetylsalicylate, and this was infused thereafter at 1 mg/min. The values for the various determinations for the 4 control periods and the 7 experimental periods were averaged and the mean values and difference are shown at the bottom of Tables I and II. Methyl ASA did not change blood pressure in this experiment and in another animal in which it was measured. There was an increase of 8.7 ml/min in C^{In} and C^{PAH} increased by 7 ml/min. There was a slight decrease in femoral artery plasma protein concentration, probably due to dilution by the infusate. For reasons discussed later, glomeru-

lar efferent arteriolar plasma protein concentration was calculated from the equation: efferent arteriolar plasma protein concentration = femoral artery plasma protein concentration/1 — (C^{In}/C^{PAH}). The equation assumes that no protein is filtered. Protein concentration in efferent arterioles is higher than that in peripheral blood due to the concentrating effect of loss of water from plasma perfusing the renal cortex into glomerular filtrate. There was a mean increase of 0.2 g/100 ml in calculated efferent arteriolar protein concentration. However in 3 experimental periods, the value was equal to or less than the mean value calculated for control periods (7.2 g/100 ml). Methyl ASA decreased V and C^{osm} slightly but there was relatively little effect on TC H_2O.

Table II shows the effect of methyl ASA on ionic excretion in the same animal described in Table I. Despite an increased filtered load of both ions, Na^+excretion decreased 68 μmoles/min and Cl^- excretion decreased 46 μmoles/min. Methyl ASA reduced Ca^{2+} excretion 1.04 μmoles/min and Mg^{2+} excretion 0.33 μmoles/min. The HCO_3^- excretion fell by 15.9 μmoles/min despite a slight increase in filtered load and some decrease in pCO_2. The latter is a factor which should inhibit HCO_3^- reabsorption and increase excretion rate. The K^+ excretion decreased by 20.5 μmoles/min. Figure 1 shows the effect of methyl acetylsalicylate on the excretion of Na^+, Cl^-, and Mg^{2} in another experiment. During control periods the excretion of Na^+ and Cl^- ranged between 100 and 160 μmoles/min. Following methyl acetylsalicylate there was a marked decrease in the rate of chloride excretion. In the last clearance period this fell to 10 μmoles /min. There was also a striking reduction in the rate of Na^+ excretion which ultimately decreased to 26 μmoles/min. Calcium and magnesium excretion ranged between 2.3 and 3.3 μmoles/min during the control period. After methyl acetylsalicylate the excretion rates of these ions fell to between 0.2 and 0.7 μmoles/min. In this experiment, there was no appreciable change in inulin clearance and in the plasma concentrations of Na^+, Cl^-, Ca^{2+} and Mg^{2+}.

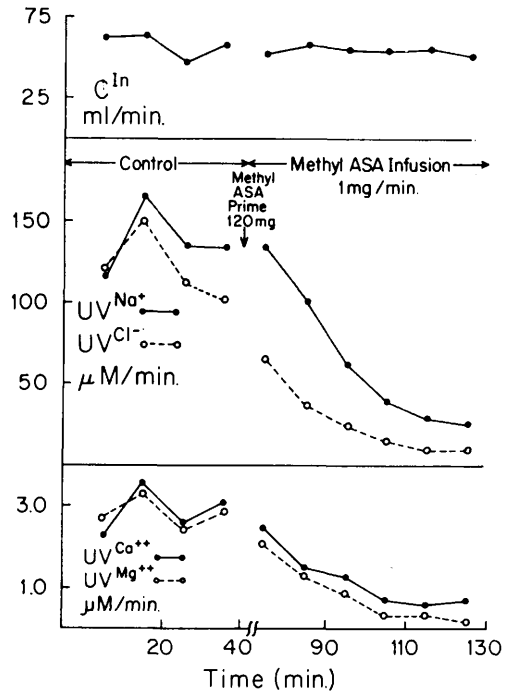
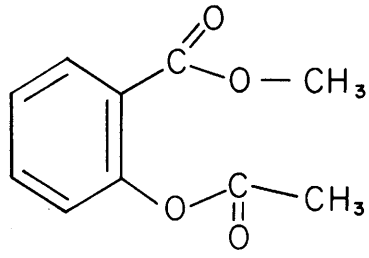


FIG. 1. Effect of methyl ASA on C^{In}, and urinary excretion of sodium (UV^{Na^+}), chloride (UV^{Cl^-}), calcium ($UV^{Ca^{2+}}$) and magnesium ($UV^{Mg^{2+}}$). There was a significant decrease in UV^{Na^+}, UV^{Cl^-}, $UV^{Ca^{2+}}$, and $UV^{Mg^{2+}}$ without change in C^{In}.

Table III summarizes all experiments. The data in Table III are presented as percentage of control rates ± SE. For each animal, a mean control value for ionic excretory rates, C^{In}, V, RPF, and TC H_2O was determined from the 4 control periods. The values for each experimental period were expressed as a percentage of the mean control. These percentages were then averaged. As shown, methyl ASA reduced sodium excretion to 57%, chloride excretion to 51% and bicarbonate excretion to 55% of control values. Excretion of calcium fell to 66% and magnesium to 69% of control. Methyl ASA had little effect on K^+ excretion, TC H_2O and urine flow. The C^{In} increased slightly and there was a 21% drop in RPF.

Table IV shows tissue concentrations of methyl acetylsalicylate in the kidneys removed at the conclusion of clearance experiments. Concentrations of methyl acetylsalicylate ranged from 13.6 to 35.4 mg/100 g of wet weight. The mean value was 24.6. Analy-

TABLE III. Summary of Effect of Methyl ASA on Renal Function and Ionic Excretion [percentage control ± standard error (SE)].



	Methyl ASA
	% Control ± S.E.
UV^{Na^+}	57 ± 2.7
UV^{Cl^-}	51 ± 1.7
$UV^{HCO_3^-}$	55 ± 2.3
$UV^{Ca^{++}}$	66 ± 2.3
$UV^{Mg^{++}}$	69 ± 4.7
UV^{K^+}	85 ± 1.6
C^{In}	109 ± 1.7
V	94 ± 1.7
$T_{C_{H_2O}}$	99 ± 3.1
RPF	79 ± 2.9

ses of plasma for methyl acetylsalicylate in these animals failed to show any detectable amounts.

Discussion. The data show that the injection and subsequent infusion of a small amount of methyl acetylsalicylate into the dog significantly decreases the urinary excretion rates of Na^+, Cl^-, HCO_3^-, Ca^{2+}, and Mg^{2+}. Table V shows the mean ± SE values during control and experimental periods of plasma Na^+, C^{In}, filtered load of Na^+ and Na^+ excretion. As shown UV^{Na+} decreased despite an increased filtered load of Na^+ delivered to the tubules. There was a similar increase in filtered loads of Cl^- and HCO_3^-. The pCO_2 either remained constant or decreased slightly. Hence the decreased excretion of univalent ions could not be attributed to decreased filtered load or increased CO_2 tension. Under these circumstances it is reasonable to conclude that methyl acetylsalicylate increased the renal tubular reabsorption of Na^+, Cl^-, and HCO_3^-. There was no change in total plasma concentrations of calcium and magnesium. However the ionic concentrations were not determined. It is possible that methyl acetylsalicylate may have decreased the filtered loads of both divalent cations by increasing their binding to plasma protein. Thus the possibility that the reduction in Ca^{2+} and Mg^{2+} excretion might have been the result of decreased filtered load cannot be entirely excluded. However it seems more resonable to attribute the observed effect to increased tubular reabsorption of both ions.

Tubular reabsorption of Na^+ occurs as the result of active transport of sodium from tubular to peritubular fluids (8), and is influenced by peritubular capillary hydrostatic pressure (9) and renal plasma flow (10, 11). As methyl ASA did not change blood pressure, it seems unlikely that it increased Na^+ reabsorption by reducing peritubular capillary pressure. Clearance and micropuncture experiments have demonstrated that reduction of renal plasma flow will increase tubular reabsorption of Na^+ (10, 11). Decrease in renal cortical plasma flow (C^{PAH}) with no change or elevation in GFR will increase filtration fraction, and the plasma protein concentration in efferent arterioles and peritubular capillaries will consequently rise. Thus peritubular capillary colloid osmotic pressure is increased which facilitates transfer of reabsorbate from the peritubular space into the vascular compartment (11).

One mechanism by which methyl ASA in-

TABLE IV. Concentration of Methyl Acetylsalicylate in Dog Kidney.

Expt.	(mg/100 g of wet wt)
1	30.8
2	35.4
3	13.6
4	22.2
5	19.5
6	25.8
Mean	24.6

TABLE V. Plasma Na^+ Concentration, Inulin Clearance, and Na^+ Excretion (UV^{Na^+}) during Control and Methyl ASA Infusion Periods.

No. of expt.	Plasma Na^+ (mmoles/liter; mean ± SE)	C^{In} (ml/min; mean ± SE)	Filt. load Na^+ (μmoles/min; mean ± SE)	UV^{Na^+} (μmoles/min; mean ± SE)
1. Control	142 ± 0.6	49.9 ± 0.5	6742 ± 60	228 ± 6.1
Methyl ASA	137 ± 0.7	54.7 ± 1.8	7494 ± 250	94 ± 6.4
2. Control	135 ± 0.7	72.4 ± 1.8	9326 ± 244	378 ± 6.9
Methyl ASA	135 ± 0.5	72.7 ± 0.6	9306 ± 71	240 ± 8.4
3. Control	136 ± 0.2	75.3 ± 2.0	9729 ± 244	315 ± 7.2
Methyl ASA	135 ± 0.4	77.2 ± 0.5	9942 ± 41	174 ± 4.8
4. Control	136 ± 0.5	63.0 ± 1.0	8355 ± 70	209 ± 1.7
Methyl ASA	135 ± 0.7	68.9 ± 0.6	8932 ± 84	166 ±16.3
5. Control	146 ± 1.5	74.2 ± 1.7	10,110 ± 326	100 ± 4.0
Methyl ASA	137 ± 1.4	87.8 ± 2.7	11,417 ± 438	74 ± 5.8
6. Control	133 ± 2.0	49.3 ± 2.0	6206 ± 174	104 ± 4.0
Methyl ASA	129 ± 0.7	57.8 ± 2.2	7106 ± 292	38 ± 5.8

creased tubular reabsorption of Na^+ was a reduction in RPF (Table III). However, in 5 of 13 clearance periods, methyl ASA produced a significant decrease in UV^{Na+} in association with an increase in renal cortical plasma flow. This is shown in Table VI. After methyl ASA, there was a mean Δ C^{PAH} of +18 ml/min, while the mean Δ UV^{Na+} was —68 μmoles/min. Table I shows that in 3 of 7 experimental periods, the calculated efferent arteriolar plasma protein concentration was equal to or less than the mean control value. Table II shows that during these same experimental periods, methyl ASA produced a significant reduction in the rate of sodium excretion. Methyl ASA then can reduce sodium excretion without decreasing renal cortical plasma flow and increasing peritubular capillary colloid osmotic pressure. The compound probably augments tubular reabsorption of Na^+ by a direct action on the transport processes of the renal tubular epithelial cell. Tissue analysis demonstrated an avid uptake of methyl ASA by kidney from plasma (Table IV). This suggested that the compound entered the tubular cell where it could affect transport mechanisms.

The increase in tubular reabsorption of Cl^-, HCO_3^-, Ca^{2+} and Mg^{2+} produced by methyl ASA probably occurred as a result of the effect of the compound on Na^+ reabsorption. Tubular reabsorption of Cl^- is a passive process and occurs as the result of active Na^+ transport (8). HCO_3^- reabsorption results from tubular secretion of H^+ which is facilitated by a cationic exchange of H^+ for Na^+ (12). Reabsorptive mechanisms for divalent cations have not been well

TABLE VI. Periods in which Methyl ASA Reduced UV^{Na^+} without Decreasing C^{PAH}.

Control		Methyl ASA		Methyl ASA—control	
C^{PAH} (ml/min)	UV^{Na^+} (μmoles/min)	C^{PAH} (ml/min)	UV^{Na^+} (μmoles/min)	C^{PAH} (ml/min)	UV^{Na^+} (μmoles/min)
124	121	132	39	+8	—82
124	121	124	33	0	—88
121	104	170	84	+49	—20
121	104	135	49	+14	—55
121	104	142	10	+21	—94
Mean				+18	—68

defined, but a close relationship between sodium reabsorption and reabsorption of calcium and magnesium has been shown (13, 14). The increased reabsorption of calcium, magnesium, chloride, and bicarbonate produced by methyl ASA is probably the result of stimulation of the sodium or a closely related transport system.

The exact mechanisms whereby methyl acetylsalicylate stimulates ionic tubular reabsorption by a direct action on the cell were not ascertained by these studies. Several possibilities should be considered. It could attach to a carrier protein and increase the number of carrier sites. It could increase the production of metabolic energy required for an active transport mechanism. Or it could increase the permeability of the luminal membrane of the cell. There appears to be a chemical specificity for the phenomenon. Table III shows the positions of the oxygen atoms in the substituent groups *ortho* to the carboxyl group of benzoic acid. These are similar to those in acetylsalicylic acid. It is proposed that the position of these oxygen atoms gives the chemical specificity for the direct effect on the renal tubular epithelial cell resulting in increased tubular reabsorption.

Summary. The effect of methyl acetylsalicylate on renal tubular ionic reabsorption was studied by clearance experiments in dogs. The compound produced a significant decrease in the rates of excretion of Na^+, Ca^{2+}, Mg^{2+}, Cl^-, and HCO_3^-. As this occurred despite an increased filtered load of the various ions, it was concluded that methyl acetylsalicylate increased tubular ionic reabsorption. One mechanism causing increased reabsorption was a decrease in RPF. However increased ionic reabsorption was demonstrable in the absence of falling RPF. Thus a second mechanism appeared to be involved, namely a direct action of the compound on the tubular epithelial transport mechanisms.

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