Use of Amine Buffer (THAM) in Treatment of Acute Salicylate Intoxication.* (26107)

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2-amino-2-hydroxymethyl-1, 3-propanediol or tris (hydroxymethyl) aminomethane (THAM), an organic buffer of low toxicity, has been widely used for correction of metabolic and respiratory acidosis(1,2,3). In both conditions, a marked diuresis of alkaline urine was observed. Since an alkaline urine enhances excretion of weak acids(4,5,6,7), it was postulated that THAM would increase renal excretion of salicylic acid following salicylate intoxication. A simultaneous correction of the metabolic acidosis which accompanies salicylate poisoning was another expected result of this therapy.

Methods. Four mongrel dogs kept on a regular diet and given free access to water before the experiment were anesthetized with 30 mg/kg B. Wt of sodium pentothal injected intravenously. Indwelling catheters were introduced into both femoral veins and arteries and urinary bladder. An intravenous infusion of isotonic sodium chloride was administered to supply approximately 3% of body weight in 30 minutes, and to produce a measurable diuresis. Priming doses of inulin and para-amino-hippurate were injected intravenously to determine glomerular filtration rate and renal plasma flow. These doses were followed by a constant intravenous infusion calculated to replace the excreted amounts of these substances. Twenty minutes were allowed to permit an even distribution of the infused inulin and PAH. The urinary bladder was then emptied by injection of air and suprapubic pressure, and urine collections were made at regular intervals; at approximately mid-time of urine collection, blood was drawn from a femoral vein. After one or 2 control periods of 10 minutes, a single dose of 100 mg/kg sodium salicylate was injected intravenously. Thirty minutes later 0.3 M THAM solution was infused simultaneously with inulin and PAH. Rate of THAM administration was 1 ml/kg/min for the first 15 minutes and 0.5 ml/kg/min for the last 15 minutes. Total dose of THAM administered was 500-800 mg/kg. Inulin and PAH infusion were continued for an additional hour. Urine collections were made at 15, 30, 60 and 90 minutes after start of THAM administration. Blood pressure and ECG were monitored at regular intervals. Each sample of urine and plasma was analyzed for total salicylates, inulin, PAH, sodium, potassium, chloride, pH, CO_2, pCO_2 and HCO_3. pH was measured within 0.005 unit. The experimental procedure was designed as described by Kaplan and Del Carmen(4). Total salicylates in blood and urine were determined by the methods described by Brodie, Udenfried and Coburn(8) and by Smith, Gleason, Stoll and Ogorzalek(9). Determination of inulin was done by the Resorcinol method(10) and PAH by the method described by Smith *et al.*(11). Specimens were analyzed for their concentrations of sodium and potassium with the Patwin Flame Photometer and for chloride with the Buchler Cotlove chloridometer(12). For determination of pH the Astrup ME-1 micro equipment with the Sanz electrode was used. The microgasometer of Kopp-Natelson was employed for determination of plasma CO_2 content; HCO_3- and pCO_2 were calculated from the Henderson-Hasselbalch equation (13).

Results. Tables I and II present results of pH, CO_2 content, salicylate and electrolyte concentration in urine and plasma during the 4 experiments. Fifteen minutes followig onset of THAM infusion, salicylate excretion in the urine increased from 723 to 2838 μg/min a 400% increase, while pH of urine rose from 7.14 to 7.59. In contrast, be-

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TABLE 1. pH, CO_2 Content, pCO_2, Salicylate and Electrolyte Concentrations and Output of Urine in 4 Dogs after Salicylate Administration (100 mg/kg) and Treatment with THAM. THAM was given at rate of .3 mM/kg for 15 min. and at rate of .15 mM/kg for next 15 min. Sample 1 is the control, samples 2 and 3 were taken 15 and 30 min. following salicylate admin.; samples 4 and 5 were measured during THAM admin.; 6 and 7 were taken 30 and 60 min. after THAM admin. Urine collections were made over entire length of each period. First figure in each column is avg of measurements. Figures in parentheses indicate range.

Period	Time, min.	Salicyl, μg/min.	Na^+	K^+	Cl^-	HCO_3^-	Urine output, ml/min.	GFR, ml/min.	pH	CO_2, μM/l	pCO_2, mm Hg
			μeq/min.								
1. Control	0		290 (217–403)	51 (28–96)	614	11.4	2.05 (1.11–4.00)	61	7.05 (6.88–7.23)	39 (13–65)	37.6 (31.0–44.1)
Salicyl inj.→	0										
2.	15	593 (430–871)	413	35	—	23.8	3.48	62	6.88	28	60.7
3. THAM infusion	30	723 (472–860)	342 (76–604)	47 (31–219)	1030	70.8 (61–81)	3.41 (2.45–5.55)	60	7.14 (6.9–7.42)	76 (67–85)	45.7 (39.1–52.2)
4.	45	1991 (1030–3670)	356 (37–835)	77 (41–104)	970	174.5 (155–195)	4.70 (2.74–7.4)	53	7.4 (7.2–7.67)	182 (165–198)	56.9 (51.0–62.8)
5.	60	2838 (1243–4824)	446 (61–837)	131 (61–189)	1000	152.5 (39–266)	7.65 (3.41–10.2)	42	7.59 (7.51–7.67)	341 (272–410)	59.8 (50.1–69.4)
6.	90	2076 (748–3519)	477 (117–770)	75 (7–128)	865	540	6.78 (4.78–10)	31	7.53 (7.48–7.62)	554	52.9
7.	120	2783 (2531–3035)	477 (144–885)	56 (19–78)	884	700	7.08 (5.23–8.60)	44	7.46 (7.37–7.55)	456	60.5

fore THAM administration, urine salicylate excretion increased from 593 to 723 μg/min, a 12% increase, while urine pH increased from 6.88 to 7.14. There was a 10-fold increase in HCO_3- excretion, while $Na+$ rose from 413 to 477 μEq/min and $K+$ from 47 to 131 μEq/min. Chloride excretion decreased from 1030 to 865 μEq/min. There was a marked diuresis with no significant changes in inulin or PAH clearance. Seventy-five minutes after THAM administration, plasma salicylate levels had dropped from 33 to 17 mg/100 ml. The highest blood pH was 7.57, CO_2 rose from 23.6 to 32.5 mM/L, HCO_3- from 22 to 31 mM/L and pCO_2 from 32.5 to 36.6 mm Hg. $Na+$, $K+$ and $Cl-$ in plasma fell slightly but consistently.

Discussion. When THAM is used in treatment of experimentally produced acute salicylate intoxication, a significant increase in salicyluria is observed. Alkalinisation of the urine which results from this therapy may be the cause of this increase in salicylate excretion(4,5,6,7). Kaplan *et al.*(4) suggested that it might have been caused by HCO_3- and salicylate ions competing for absorption by the renal tubules. After finding that urine virtually free from HCO_3- had a higher level of salicylate when its pH was more alkaline, Kaplan also suggested that urinary pH at least partly determines rate of excretion of salicylate. Gutman *et al.*(5) and Schachter *et al.*(6) found that in man salicylate can be filtered at the glomerulus and reabsorbed and secreted by the renal tubules. Probenecid, which inhibits tubular activity, reduced the clearance of salicylate independently of urine pH, probably by inhibition of active tubular transport. When urine pH was below 6.0, there were indications of glomerular filtration and tubular reabsorption of salicylates. When urine pH was greater than 7.4 ratio of the clearance of salicylate to glomerular filtration rate (CFrS/CF) was greater than 1.0. When urine pH was kept above 7.50 by administering $NaHCO_3$ and probenecid simultaneously, a decrease in clearance of salicylate was found, supporting the belief that salicylate is actively secreted by the tubules. Weiner, Washington and Mudge(7) assessing

TABLE II. Salicylate, Electrolyte Conc., pH, pCO_2 and CO_2 Content of Plasma in 4 Dogs after Salicylate Admin. (100 mg/kg) and Treatment with THAM; Blood Samples Were Taken in Middle of Each Period. First figure in each column is avg of all measurements. Figures in parentheses indicate range.

Period	Time, min.	Salicyl, mg/100 ml	Na^+ (meq/l)	K^+ (meq/l)	Cl^- (meq/l)	HCO_3^-, mM/l	pH	Content CO_2, mM/l	pCO_2, mm Hg
1. Control		0	153 (148–155)	3.2 (2.4–3.8)	114	19.9 (19.1–21)	7.34 (7.28–7.37)	21.0 (20.1–22.1)	37.8 (37–42.6)
2. Salicyl→ inj.	0								
2.	15	30 (25–37)	151	3.5	—	22.1	7.27	23.6	49.7
3. THAM infusion	30	33 (22–54)	150 (145–154)	3.5 (3.2–3.7)	113	20.9 (18.1–27.2)	7.42 (7.37–7.57)	21.9 (19.0–28.1)	32.6 (30.6–34.9)
4.	45	29 (19–47)	140 (134–146)	3.6 (3.4–3.8)	103	27.6 (24.3–34)	7.52 (7.50–7.54)	28.9 (25.2–35.3)	34.8 (31.1–41.1)
5.	60	22 (16–28)	137 (132–142)	3.4 (3.1–3.8)	101	31.4 (27.8–35.7)	7.57 (7.51–7.67)	32.5 (28.9–36.9)	35.3 (26.8–40.2)
6.	90	19 (16–24)	146 (145–147)	2.8 (2.7–3.0)	102	28.0 (23.3–30.6)	7.51 (7.41–7.64)	29.1 (24.4–31.9)	36.7 (28.9–43.2)
7.	120	17 (10–23)	144 (141–150)	2.7 (2.6–3.0)	106	26.0 (21.4–28.9)	7.50 (7.45–7.53)	27.4 (22.3–30.1)	35.0 (31.3–39.8)

the relative roles of urinary pH and urine volume, found that a CFrS/CF ratio significantly greater than 1.0 with a urine pH more acid than that of blood could be produced when the ratio of volume of urine to glomerular filtration rate $\left(\frac{V}{GFR}\right)$ was .09. These authors concluded that salicylate was secreted by the tubule after observing that salicylate elimination in the urine was depressed by other substances like PAH and acetazolamide, which are also secreted by the tubules, and by compounds like probenecid that inhibit other tubular transport mechanisms. It was postulated that a passive ion trapping mechanism would cause reabsorption of salicylic acid.

Regardless of the final proof of the mechanism by which salicylate is reabsorbed or secreted, the present experiments indicate that both an alkaline urine and an increase in urine formation enhance elimination of salicylate by the kidney. For instance, in Exp. 4 the highest salicylate urinary excretion (4824 μg/min) was accompanied by the largest diuresis (10.2 ml/min). The marked salicylate output was attained although urinary pH (7.67) was not as high as the pH reported by others(4,7). Furthermore, protein binding of salicylate may be pH dependent: the lower the pH, the greater the binding. It is therefore important to have an alkalinization of body fluids so that this binding be minimal (personal communication). THAM, by producing an alkalosis of intra- as well as extra-cellular fluid, will therefore limit protein binding of salicylate and favor its renal excretion. For the same reason, acetazolamide, which produces a metabolic acidosis, may not be the best agent to combat salicylate intoxication, although acetazolamide induces excretion of an alkaline urine and has been advocated for treatment of salicylate poisoning(14,15). When THAM was first employed in treatment of experimentally produced metabolic and respiratory acidosis and in man, its possible usefulness in acute salicylate poisoning was apparent. The results of these experiments confirm this hypothesis. Since THAM has been found to be safe in humans(3,16,17) in doses similar to those used in the present experiments, it should be beneficial in treatment of infants and children suffering from salicylate poisoning. THAM has already been used in one such instance(18).

Summary. The effectiveness of tris (hydroxymethyl) aminomethane (THAM) in increasing salicyluria after intravenous injection of sodium salicylate was tested in experiments on 4 dogs. A marked drop of salicylate in plasma and increase in its excretion in urine was demonstrated. These ex-

periments also indicate that maximum excretion of salicylate by the kidney will occur when an elevation of body fluid pH and urine pH are accompanied by a marked diuresis. THAM, which promotes these changes, may be useful in treatment of salicylate poisoning in man.

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Effect of Internally Deposited Radioisotopes upon Blood Vessels of Cortical Bones.* (26108)

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Recent reviews by investigators dealing with internally deposited radioisotopes in bone state there is no evidence that injury to the bone was secondary to injury to small blood vessels directly supplying the bone(1, 2). They conclude that injury to the various cellular constituents of the bone appears to be a direct effect of the radiation upon them. This conclusion is based on the study of bones from radium patients in which activity is found throughout bone, and the alpha particles appear to be killing osteocytes directly.

In the present study we demonstrate that much of the damage in cortical bone is due to vascular occlusion. These studies have utilized dogs containing radium, plutonium or radiothorium in which a new technic of vascular injection of India ink-gelatin has been used to demonstrate the disturbance in blood supply. Unlike radium, which is distributed throughout bone, plutonium and radiothorium are localized on bone surfaces and the short range of the alpha particles located about haversian canals allows the major portion of the haversian systems and all the interstitial lamellae to be free of activity. Thus, if massive devitalization of bone is observed, it cannot be related to direct killing by alpha rays.

Materials and methods. The materials used were the mid-diaphyses of tibiae from 4 normal and 23 beagles that died or were sacrificed from 1 to 1724 days following administration of radium-226, plutonium-239 and

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