no explanation for the increasing activity after the fifteenth year.

Summary. The tissue distribution of Cs^{137} after chronic administration is markedly similar in mice and dogs. The highest concentration is found in muscle, while fat, blood, skin, and bone have lower concentrations. Other tissues tend to conform to the concentration in the whole body, which is less than in muscle and more than in fat. Differences in whole bone concentration are due in part to the state (red or fatty) and quantity of marrow; for example, dog ribs have a higher concentration than dog femurs.

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Received March 11, 1964. P.S.E.B.M., 1964, v116.

Urinary Excretion of THAM Citrate Orally Administered.* (29253)

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Tris(hydroxymethyl)aminomethane (THAM), a weak organic base of low toxicity, has been widely used both experimentally and clinically in an intravenous solution to correct respiratory and metabolic acidosis (1,2,3). Although the intravenous infusion of THAM must be performed with caution, it is the most effective mode of administration for rapid and stochiometric titration of fixed acid *in vivo.* Indeed, when administered orally, some systemic alkalinization is produced, but it is accompanied by marked diarrhea(4). In addition, THAM is very unpalatable, and in the few cases reported of oral administration, a gastric tube had to be used. Administration of THAM crystals in gastric coated capsules is also accompanied in animals and man by diarrhea(5).

It has also been observed that THAM, titrated with acid to a less alkaline pH, is not as irritating to the tissues at the site of infusion(6). To increase gastric and intestinal tolerance to THAM administration, therefore, tests were made on THAM salts of a number of weak acids. THAM citrate was selected because it is stable and very soluble in water. A mixture was made containing 20% of THAM, 4.2% of citric acid, a cherry-flavored syrup and 2% alcohol. The pH of the mixture is 8.50 at 37°C and it retains 70% of its titrating activity. Each ml contains 1.3 mM of un-ionized THAM, an amount of base which compares well with standard bicarbonate solutions used for treatment of renal acidosis(7). The purpose of the present study was to determine the effect and mode of action of THAM citrate administered orally.

Method. Three series of experiments were performed on 4 normal mongrel dogs. The animals were given a meat diet and water *ad libitum.* In the first series of experiments 3

* This work was supported in part by grants from N.I.H. and from Abbott Laboratories.

TABLE I. Changes in pH, "Standard Bicarbonate" and "Base Excess" of 3 Dogs Fed with THAM Citrate (5 mM/kg).

	Days	Dog 1* pH	Dog 1* B.E.	Dog 1* Std. HCO_3^-	Dog 2† pH	Dog 2† B.E.	Dog 2† Std. HCO_3^-	Dog 3‡ pH	Dog 3‡ B.E.	Dog 3‡ Std. HCO_3^-
	1	7.38	−4.2	20.0	7.25	−12.3	15.0	7.34	−4.2	20.0
THAM§	2	7.42	+3.2	25.2	7.32	− 5.3	19.2	7.35	+ .6	23.3
	3	7.40	+3.8	24.5	7.36	− 1.8	21.6	7.38	+1.0	23.5
	4	7.42	+4.0	25.5	7.33	− 4.3	20.0	7.41	+3.0	25.0
	5	—	—	—	7.38	− 2.2	21.4	7.36	− .9	22.3
	8	7.34	−4.0	20.2	7.31	− 6.7	18.4	7.32	−2.8	21.0

* Total THAM administered: 320 mM. † Total THAM administered: 340 mM. ‡ Total THAM administered: 285 mM.

§ THAM citrate, 5 mM/kg/day.

TABLE II. Effect of THAM Citrate on Urine pH, CO_2 Content and HCO_3^-.

	Days	Dog 1 pH	Dog 1 CO_2 content mM/l	Dog 1 HCO_3^-* meq/l	Dog 2 pH	Dog 2 CO_2 content mM/l	Dog 2 HCO_3^- meq/l	Dog 3 pH	Dog 3 CO_2 content mM/l	Dog 3 HCO_3^- meq/l
	1	6.07	2.65	1.30	5.81	3.12	1.18	6.42	4.86	3.24
THAM†	2	7.08	39.20	35.67	7.10	42.31	38.16	7.24	41.28	38.34
	3	7.35	67.48	64.11	7.35	59.75	56.20	7.42	62.40	59.22
	4	7.33	65.40	61.48	7.46	63.46	61.00	7.45	68.54	65.92
	7	6.34	5.37	3.38	6.12	4.36	2.18	5.96	3.87	1.71

* Estimated from pH and total CO_2 content according to Peters and Van Slyke, *Quantitative Clinical Chemistry: Methods*, Vol. 11, Williams and Wilkins, 1932.

† THAM citrate, 3.3 mM/kg/day.

dogs received daily, for 5 consecutive days, 5 mM per kg/bodyweight (3 ml) of THAM citrate, given in 2 doses, one in the morning and one in the afternoon. Control venous blood samples were collected before the start of the experiment and at 24 hour intervals following administration of the second dose of THAM. A sample was also taken 3 days after THAM citrate administration had been discontinued. The samples were analyzed for standard bicarbonate and base excess according to the method of Astrup and Siggaard-Andersen(8).

In a second series of experiments 4 dogs received daily for 3 consecutive days 3.3 mM/kg of THAM citrate administered in 2 doses. The dogs were kept in metabolic cages and urine was collected every 24 hours for 7 consecutive days. The amount of THAM excreted in the urine was determined by the method of Rosen(9). In addition, tracer amounts of C^{14}-tagged THAM were added to the THAM citrate given to one of the dogs. and excretion of THAM in urine and feces was calculated from the C^{14} counts according to a technique previously described (10). To study pH changes in the urine, 3 of the dogs were anesthetized with thiopental (13 mg/kg) immediately before the second dose of THAM citrate, and a sample of urine was collected by catheter. pH was measured with a Radiometer pH meter and an Astrup micro glass electrode. Total CO_2 content of urine was determined with a Natelson microgasometer and bicarbonate was estimated from the pH and total CO_2 content.

In a third series of experiments 3 dogs were given daily 1.6 to 3.3 mM/kg of THAM citrate syrup on 5 consecutive days each week for a period of 3 months. 1.6 mM/kg was administered for the first month, 2.4 for the second month and 3.3 for the third month. The animals were weighed each week. After 3 months, blood samples were collected and measurements were made of hemoglobin, platelets, and red and white blood cell count.

Results. (Tables I-IV). THAM citrate

TABLE III. Urinary Excretion of THAM During and Following 3 Days of Oral Administration of 3.3 mM/kg/Day of THAM Citrate.

	Days	Dog 1* (wt 10.9 kg) Urine vol	mM THAM excreted	% total recovered	Dog 2† (wt 10.0 kg) Urine vol	mM THAM excreted	% total recovered	Dog 3‡ (wt 9.0 kg) Urine vol	mM THAM excreted	% total recovered
THAM§	1	312	24.2	67	320	22.7	69	165	18.8	63
	2	180	16.6	56	585	24.1	71	215	21.6	68
	3	306	26.0	61	340	22.4	70	385	22.9	71
	4	150	3.8	65	232	3.4	74	27	1.4	73
	5	330	4.1	69	615	5.1	79	240	3.4	77
	6	267	2.6	71	580	—	—	280	2.8	80
	7	485	3.6	74	210	—	—	95	.8	81
Total:		2,030	80.9	74	2,882	77.7	79	1,407	71.7	81

* Total THAM administered 109 mM. † Total THAM administered 99.2 mM. ‡ Total THAM administered 89.3 mM.

§ THAM citrate, 3.3 mM/kg/day.

TABLE IV. C^{14} Activity in Urine and Feces Following Oral Administration of THAM Citrate with Tracer Amount of C^{14} Tagged THAM.

	Days	Dog 4 (wt 8.8 kg) Urine: THAM exc., mM	Urine: % total THAM admin.	Feces: THAM exc., mM	Feces: % total THAM admin.	THAM exc., % total admin.
THAM*	1	19.6	67.4	3.4	11.7	79.1
	2	19.1	66.5	2.7	10.5	77.0
	3	22.1	69.6	.9	8.0	77.6
	4	1.2	71.0			79.0
	5	.8	71.9			80.0
	6	.2	72.2			80.2
	7	.1	72.3			80.3

* THAM citrate, 3.3 mM/kg/day. Total THAM administered, 87.3 mM.

administration was accompanied by an increase in the "standard bicarbonate" and "base excess" of the plasma, and a shift of the pH of the urine collected during the 24 hours following THAM administration from 6.1 to 7.14. A marked increase in CO_2 and HCO_3^- content of the urine was also observed. Urinary excretion of THAM amounted in one dog to 18% of the administered dose after 6 hours, and in 4 animals averaged 67% after 24 hours, 71% after 48 hours and 74% after 72 hours. Determinations of THAM by the Rosen method or by C^{14} counting gave similar results; 9-11% of the dose administered was recovered in the feces after 24 hours. Fifteen minutes after administration of THAM, radioactivity was detected in the plasma (0.7 mM/l). THAM concentration in the plasma reached a plateau 2 hours later (1.2 mM/l) and decreased progressively thereafter (0.3 mM/l after 6 hours). Twenty-four hours after administration of the THAM citrate, no radioactivity was detected in the plasma while there were still C^{14} counts present in the urine (Fig. 1).

Dogs which had been given a daily dosage of 1.6 to 3.3 mM of THAM citrate on 5 consecutive days each week for a period of 3 months showed no apparent ill effects. At the end of this period their body weight was maintained or increased. Hemoglobin, platelets, red and white blood cell counts were normal.

Discussion. Urinary excretion of THAM citrate administered orally indicates that this compound is absorbed through the intestinal mucosa and subsequently eliminated by the kidney. Over a 72 hour period, the combined recovery in urine and feces was 16% less than the dose administered. However, the difficulty in obtaining complete collection of urine and feces could account for the major part of this discrepancy. When THAM was administered intravenously, under conditions

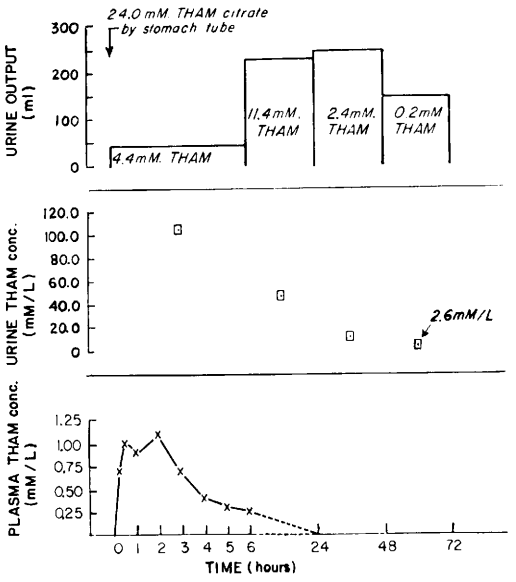

FIG. 1. Plasma and urine THAM levels and urine output in one dog following administration of 24.0 mM of THAM citrate containing tracer amounts of C^{14}-tagged THAM.

of a CO_2 load so that blood pH remains normal(11), urinary excretion was more rapid: 30-35% of the injected THAM was excreted in the urine at the end of infusion and 71% after 3 hours. However, the percentage of the total dose excreted was comparable: 74% after 24 hours, 81% after 48 hours, and up to 90% after 72 hours.

The mechanism of urine alkalinization by THAM citrate given orally seems to be similar to that of urine alkalinization by THAM base administered intravenously. THAM eliminated by the kidney is mostly in the ionized form ($R–NH_3^+$) and is accompanied by an increased HCO^- excretion. An important fraction of the HCO_3^- eliminated in the urine results from the increased plasma HCO_3^- produced by the titration of free CO_2 by THAM ($R—NH_2+CO_2+H_2O \rightleftharpoons R—NH_3^+ + HCO_3^-$). In the present experiments the increase in HCO_3^- excretion was parallel to the THAM elimination. The lack of water diuresis observed contrasts with the marked diuresis present when THAM is administered intravenously. However, in the latter case the blood levels of THAM were much higher and a large water load was also administered.

The mechanism of the transfer of THAM across the gastro-intestinal tract remains to be thoroughly investigated. Two factors should impede non-ionic diffusion of THAM: the low lipid solubility of this compound and the fact that at the pH of the gut (7.0) 85% of the THAM would be ionized. Indeed, investigators of drug absorption(12) have shown that the epithelium of the small intestine like that of the stomach allows ready penetration of lipid soluble, un-ionized molecules and impedes the penetration of ionized moieties. Citrate is in all probability also absorbed from the intestine into the blood stream, metabolized and transformed into CO_2. However, in view of the increased basicity of body fluids produced by THAM, significant amounts of citrate could also be excreted by the kidney as shown by Simpson(13).

Since there were no apparent ill effects with long term administration of THAM citrate, this therapy could be of clinical usefulness in cases where Na intake must be restricted (hypertension, cardiac failure) and alkalinization of the urine is desirable (uricemia, hyperchloremic acidosis), and possibly in renal tubular acidosis.

Summary. Administration of 20% THAM citrate (pH 8.50) to dogs on a meat diet results in increase of the base content of the blood and in the production of an alkaline urine. Between 60 and 70% of the THAM administered is excreted daily in the urine indicating intestinal absorption. This treatment which was well tolerated over a 3-month period could have useful applications in clinical medicine.

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Received January 17, 1964. P.S.E.B.M., 1964, v116.

Collagenolytic Activity of Mammalian Pancreas.* (29254)

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Several descriptions of the collagenolytic activity of the pancreas have been published. Ziffren and Hosie(1) originally claimed that pancreatic juice could solubilize collagen from Achilles tendon, but Straumford and Hummel(2) found that this activity was completely suppressed by soybean trypsin inhibitor. Banga and Balo(3) reported that pancreas contained a collagen solubilizing enzyme which attacked the "mucoprotein" cement maintaining the integrity of collagen bundles. These workers(4) later characterized this enzyme as "collagen mucoproteinase" and indicated that the products of the action of this enzyme were not dialyzable. Collagen mucoproteinase apparently does not attack peptide bonds, but rather attacks ester bonds.

We reported that saline extracts of whole pancreas could both inhibit the fiber forming ability and decrease the viscosity of solutions of salt soluble collagen in the presence of excess soybean trypsin inhibitor(5). This activity could be partially purified by extraction of the pancreas at pH 8.6(6). In the presence of soybean trypsin inhibitor, these extracts could release ninhydrin reacting materials from salt soluble collagen, and could reduce the intrinsic viscosity of these collagen solutions from 14dl/g to about 6dl/g while the optical rotation of collagen was decreased from −365 to −260°(6). This activity was not dependent upon the presence of calcium and was maximal at pH 5.5(6).

This paper describes the results of some critical experiments concerning the nature of this collagenolytic activity of pancreas.

Materials and methods. Salt soluble collagen was prepared from acetic acid extracts of calf skin as we have described previously (6). This material was purified by repeated solubilization in 0.5 M acetate (pH 5.5) and precipitation by dialysis against water. The final product had an intrinsic viscosity of 14.3dl/g and an optical rotation of −370° and contained 18.6% nitrogen(7) and 13.6% hydroxyproline(8).

Precipitated collagen was prepared from clarified acetic acid (0.5 M) extracts of bovine Achilles tendon by addition of $CaCl_2$ to a concentration of 0.5 M. The resulting precipitate was washed extensively with $CaCl_2$ and then with water.

Insoluble collagen was prepared by washing minced Achilles tendon in a Waring blendor for 3 minutes with 0.5 M acetate buffer pH 5.5 at 4°C. The tendon suspension was allowed to stand overnight in the cold. After centrifugation at 24,000 × *g*, the residue was then washed repeatedly with water and dried *in vacuo*.

Crude pancreatic collagenolytic activity was prepared by extracting 100 *g* of whole, defatted and desiccated pancreas (Bridase) in the cold in a Lourdes Omni-mixer for 5 minutes with 1500 ml of 0.1 M carbonate buffer, pH 8.6. After centrifugation, the clear extract was dialyzed and lyophilized.

Crystalline trypsin and soybean trypsin inhibitor were obtained from the Worthington Co. (New Jersey).

* Supported in part by Grant-in-Aid from N.I.H., U.S.P.H.S.