3. Leveille, G. A., Hanson, R. W., Canad. J. Physiol. Pharmacol., 1965, v43, 857.
4. ———, J. Lipid Res., 1966, v7, 46.
5. ———, Canad. J. Physiol. Pharmacol., 1966, v44, 275.
6. Leveille, G. A., J. Nutrition, 1966, in press.
7. Cohn, C., Joseph, D., Bell, L., Frigerio, N. A., Proc. Soc. Exp. Biol. and Med., 1964, v115, 1057.
8. Cohn, C., Joseph, D., Bill, L., Oler, A., Am. J. Physiol., 1963, v205, 71.
9. Umbreit, W. W., Harris, R. H., Stauffer, J. F., Manometric Techniques, 4th ed., Burgess Publishing Co., 1964, p132.
10. Herrera, M. G., Renold, A. E., Biochim. Biophys. Acta, 1960, v44, 165.
11. Gellhorn, A., Benjamin, W., Science, 1964, v146, 1166.
12. ———, Ann. N. Y. Acad. Sci., 1965, v131, 344.
13. Benjamin, W., Gellhorn, A., J. Lipid Res., 1966, v7, 285.
14. Goodman, H. M., Am. J. Physiol., 1964, v206, 129.
15. Krahl, M. E., ibid., 1964, v207, 1169.

Received July 26, 1966. P.S.E.B.M., 1966, v123.

Effects of "Net Acid" Excretion on Acid-Base Status in Dogs Following 2-Amino-2-Hydroxymethyl-1,3-Propanediol (THAM) Administration.* (31533)

MERRILL N. CAMIEN, LEO M. SMITH,† AND DANIEL H. SIMMONS
Cedars-Sinai Medical Research Institute; Division of Medicine, Cedars-Sinai Medical Center; Departments of Medicine and Physiology, University of California Medical Center, Los Angeles

It is generally recognized that an important function of the kidneys is to contribute to the maintenance of pH homeostasis by excreting titratable acid (TA) and ammonium ion. According to current concepts, the principal effect of TA excretion is to promote the generation of bicarbonate from hydrated CO_2, and since bicarbonate excretion necessarily nullifies this effect, it has been usual, as pointed out by Elkinton, *et al*(1), to calculate "net acid" excretion in terms of urinary bicarbonate as well as of TA and ammonium ion from the expression, "net acid" = TA + NH_4^+ − HCO_3^-. A widely accepted interpretation of the effects of ammonium ion excretion is that although ammonium ion is not measurable by titration with base to blood pH, it nevertheless carries an excess proton and may therefore be regarded as equivalent to urinary buffers in which the excess protons are directly measurable by titration and are therefore accounted for as TA. If this interpretation were extrapolated to other protonated, and similarly not-titratable urinary products, it would appear that the latter, as well as ammonium ion, should be taken into account in the "net acid" expression. Thus, Nahas *et al*(2), in calculating "hydrogen ion" excretion following 2-amino-2-hydroxymethyl-1,3-propanediol (THAM) infusion in dogs, employed the expression, $UV_{H^+} = UV_{NH_4^+} + UV_{TA} + UV_{RNH_3^+} - UV_{HCO_3^-}$, in which RNH_3^+ designates protonated THAM, and regarded earlier calculations reported from the present authors' laboratory(3) as erroneous for not similarly considering RNH_3^+ as a component of "acid" excretion.

On the other hand, it is well known that ammonium ion is readily metabolizable, leading to production of hydrogen ion and urea from the net reaction, $2\ NH_4^+ + CO_2 \rightarrow 2\ H^+ + (NH_2)_2CO + H_2O$, and it follows that the effects of ammonium ion excretion might alternatively be interpreted as owing to elimination of a metabolic precursor of hydrogen ion rather than of immediately available hydrogen ion. If this alternative interpretation were the correct one, then excretion of RNH_3^+, since it is not metabolizable, could not be equivalent to ammonium

* Supported in part by USPHS Grant AM-08523 from Nat. Institute of Arthritis and Metab. Dis., and in part by USPHS Grant HE-08075 from Nat. Heart Inst.

† Supported by Student Research Associate Program, California Heart Assn., Summer, 1964.

ion excretion, and the formulation employed by Nahas *et al*(2) would be incorrect. Data reported in the present communication support this alternative interpretation of the effects of ammonium ion excretion by demonstrating that RNH_3^+ excretion, in contrast to NH_4^+ excretion, does not effect a shift in systemic acid-base status in the direction of decreased acidity.

Methods. Ten experiments were conducted on female dogs anesthetized with sodium pentobarbital (28 mg/kg plus additional doses as needed). Ventilation was maintained constant with a Harvard respiration pump at 30 respirations per minute and a tidal volume of approximately 10 ml/kg, adjusted to maintain arterial pH at 7.38-7.42. Deep body temperature was monitored with an esophageal thermistor and maintained near 38° by applying heat as required with a heating pad over the chest and abdomen. Indwelling catheters were introduced into the jugular vein and bladder, and a needle with an indwelling stylet was placed in the femoral artery. A priming dose of 1 ml/kg of 1.91 *M* mannitol was administered and followed by infusion of 0.27 *M* mannitol at 0.3 ml/min/kg. Surgical silk was placed around both ureters through a flank incision and was tied loosely to allow the ureters to remain functional.

Urine samples were collected anaerobically at the end of each of two 30-minute control periods. Anaerobic arterial blood samples were collected at the midpoint of each period and at the end of the second control period. Experimental periods were begun after 2 consecutive control periods in which blood pH remained constant within 0.03 pH unit.

At zero time, the mannitol infusion was terminated and 0.4 *M* THAM was infused at 0.3 ml/min/kg for 1 hr. Blood pH, P_{CO_2} and base excess were monitored at 20-minute intervals, employing the analytical procedures of Jørgensen and Astrup(4).

In 5 of the experiments the ureters were tied off at the end of the final control period (zero time), and left closed for the duration of the experiment. In the 5 remaining experiments the sham ligatures were left open during the entire experiment. In these experiments, mannitol infusion was resumed at half the initial rate (*i.e.*, at 0.15 ml/min/kg) to maintain an adequate urine flow. The bladder catheter was clamped at the beginning of each infusion period and the bladder was emptied at 30-minute intervals (a 2 ml sample was collected anaerobically at each interval for pH determinations). Anaerobic blood samples were collected at the mid-point of each period and at the end of the THAM infusion. Renal "net acid" ($TA + NH_4^+ - HCO_3^-$) and urinary THAM were measured by the method of Chapman, *et al*(5). Urinary RNH_3^+ (ionized THAM) and RNH_2 (non-ionized THAM) were calculated from the total THAM and urine pH values as described by Nahas *et al*(2). "H^+" excretion (according to the formula of Nahas *et al*) was calculated as urinary "net acid" plus urinary RNH_3^+.

Results. Mean cumulative excretions of protonated THAM (RNH_3^+), "net acid," and the sum of "net acid" and RNH_3^+ by the experimental (ureters open) animals are shown in Fig. 1, in which it may be seen that whereas the observed "net acid" values were negative (indicative of "net base" excretion), the corresponding values for the sum of "net acid" and RNH_3^+ were positive. All cumulative excretion values for the control (ureters closed) animals were, of course, necessarily zero.

Mean changes in blood pH and blood base excess for both groups of animals are shown in Fig. 2 and 3, respectively. Both blood pH and blood base excess rose markedly during the THAM infusion and fell markedly immediately after terminating the THAM infusion in all animals (Fig. 2 and 3); however, the fall in both blood pH and blood base excess was significantly more marked in the experimental animals (ureters open) than in the controls (ureters closed).

Raw experimental values corresponding to the derived data shown in Fig. 1 through 4 are compiled in Tables I and II.

Discussion. The use of control animals with closed ureters and the maintenance of controlled respiration in all animals in the present experiments permits a direct evaluation of the effects of renal "net acid" excretion on changes in acid-base status of the blood. The latter changes in the experimental (ureters

open) animals are seen in Fig. 2 and 3 to be definitely in the direction of increased acidity relative to the control (ureters closed) animals, and the observed cumulative "net acid" excretion values for the experimental animals (since the values are negative) are clearly consistent with this outcome. It is also evident, however, that there is a marked cumulative excretion of RNH_3^+ (protonated THAM) during the period of relatively increasing acidity in the blood of the experimental animals (Fig. 1) and that the cumulative excretion values for the sum of "net acid" and RNH_3^+ (Fig. 1) are therefore appreciably positive during this period. The latter excretion values, which may also be

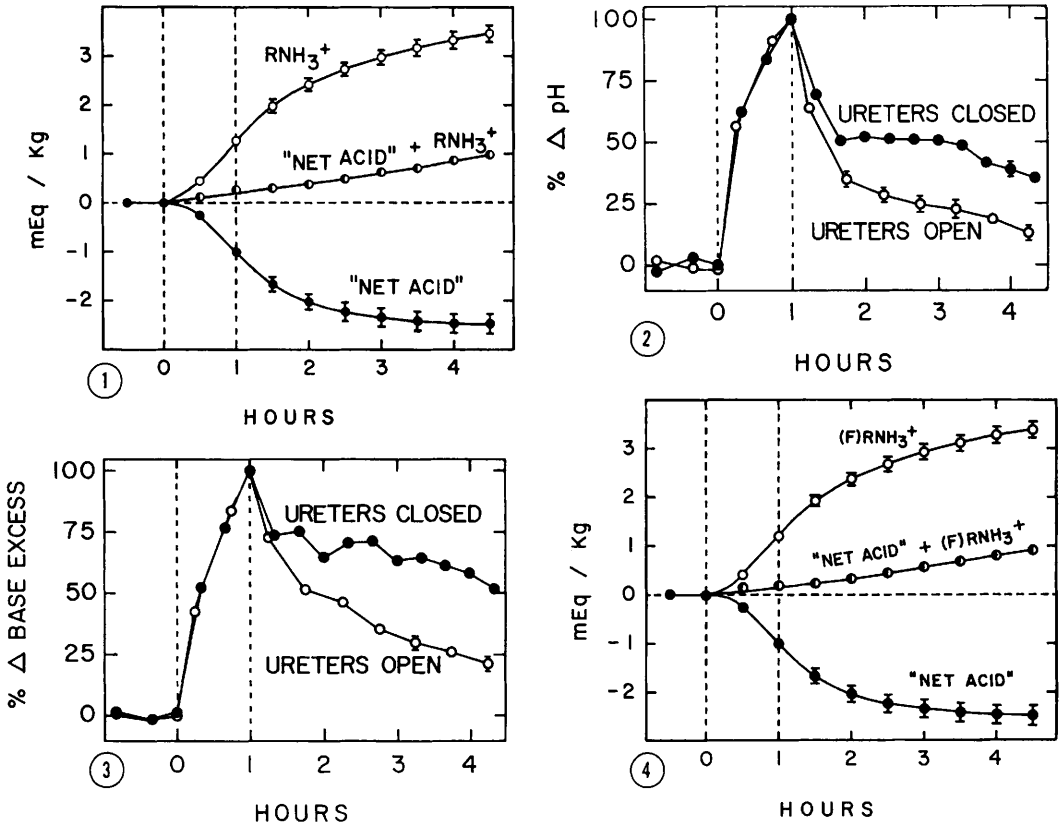

FIG. 1. Mean cumulative excretions (excluding base-line excretion) of THAM ion (RNH_3^+), "net acid" and "net acid" + THAM ion for the experimental group following THAM infusion over the interval bracketed by vertical broken lines (0-1 hr). The magnitudes of the standard errors of the mean values are either less than the diameters of the plotted circles or indicated by the vertical spans drawn through the circles.

FIG. 2. Mean changes in blood pH calculated as % of maximal change for experimental (ureters open) and control (ureters closed) dogs following THAM infusion over the interval bracketed by vertical broken lines (0-1 hr). The mean maximal change indicated by 100% on the vertical scale was + 0.22 pH unit. The magnitudes of the standard errors of the mean values are indicated as in Fig. 1.

FIG. 3. Mean changes in blood base excess calculated as % of maximal change for experimental (ureters open) and control (ureters closed) dogs following THAM infusion over the interval bracketed by vertical broken lines (0-1 hr). The mean maximal change indicated by 100% on the vertical scale was + 14.5 mEq of base excess per liter. The magnitudes of the standard errors of the mean values are indicated as in Fig. 1.

FIG. 4. Mean cumulative excretions (excluding base-line excretion) of $(F)RNH_3^+$ (filtered THAM ion), "net acid" and "net acid" + $(F)RNH_3^+$ for the experimental group following THAM infusion over the interval bracketed by vertical broken lines (0-1 hr). The magnitudes of the standard errors of the mean values are indicated as in Fig. 1.

TABLE I. Blood Parameter Changes Induced by THAM Infusion.

Elapsed time in min	Experiment No. 1	2	5	9	12	3	4	7	8	10	1	2	5	9	12	3	4	7	8	10
	Base excess, mEq/l										pH									
	Closed ureters					Open ureters					Closed ureters					Open ureters				
—50	—2.7	1.0	2.8	—3.0	4.4	1.1	—1.0	4.0	3.2	.7	7.36	7.36	7.45	7.40	7.42	7.42	7.42	7.35	7.39	7.42
—20	— .8	—1.8	2.8	—1.6	3.5	—2.0	— .8	4.2	2.9	.2	7.39	7.36	7.45	7.42	7.41	7.42	7.41	7.35	7.43	7.40
0	—1.7	— .4	2.8	—1.3	5.1	— .4	— .9	4.3	3.5	—.4	7.38	7.37	7.45	7.40	7.42	7.40	7.41	7.35	7.43	7.39
15						2.9	5.7	10.6	10.8	7.8						7.49	7.57	7.49	7.56	7.52
20	5.1	5.1	11.6	5.6	13.0						7.52	7.44	7.61	7.53	7.57					
40	8.7	8.3	14.9	10.0	15.5						7.56	7.50	7.67	7.56	7.60					
45						8.6	12.4	16.0	17.2	13.0						7.59	7.65	7.52	7.63	7.63
60	11.4	9.8	19.0	15.4	19.4	10.6	14.5	18.4	18.6	17.0	7.59	7.53	7.71	7.60	7.62	7.61	7.67	7.55	7.66	7.62
75						6.8	8.2	14.9	15.8	13.0						7.75	7.61	7.44	7.58	7.54
80	8.7	6.1	14.6	10.8	16.2						7.53	7.47	7.62	7.54	7.54					
100	8.7	8.1	14.3	10.0	15.5						7.48	7.47	7.59	7.49	7.51					
105						6.0	6.0	11.0	11.8	8.7						7.49	7.55	7.40	7.49	7.46
120	7.0	5.0	13.8	9.1	15.5						7.48	7.46	7.61	7.50	7.51					
135						4.0	6.1	9.9	11.8	8.7						7.47	7.52	7.37	7.50	7.46
140	7.5	7.4	14.6	9.1	14.9						7.48	7.46	7.60	7.50	7.51					
160	6.9	7.4	15.2	9.1	15.5						7.48	7.46	7.58	7.50	7.52					
165						2.8	5.0	7.7	11.0	6.1						7.46	7.52	7.35	7.49	7.46
180	7.1	5.5	14.2	8.0	14.0						7.49	7.45	7.60	7.49	7.51					
195						2.0	5.1	7.3	10.5	3.7						7.43	7.51	7.35	7.49	7.48
200		7.0	14.6	7.8	13.0							7.45	7.58	7.49	7.52					
220			13.0	7.9	14.0								7.56	7.48	7.51					
225						2.8	4.8	6.9	6.2	4.5						7.43	7.49	7.37	7.46	7.46
240			13.4	7.2	12.8								7.54	7.47	7.52					
255						2.8		5.2	5.7	5.9						7.41		7.36	7.47	7.46
260			11.9	7.2	11.4								7.54	7.47	7.50					
280			12.6										7.53							

TABLE II. Urine Parameter Changes Induced by THAM Infusion.

Time interval in min	Urine flow, ml/min			Urine pH			Experiment No.* Excreted, μmol/min/kg Total THAM			NH_4^+			"net acid"		
	7	8	10	7	8	10	7	8	10	7	8	10	7	8	10
−60 to −30	4.33	3.04	3.81	7.22	7.20	7.12	.0	.0	.0	.44	.32	.40	− 3.6	− 2.4	− 2.8
−30 to 0	4.87	4.34	4.27	7.17	7.08	7.21	.0	.0	.0	.57	.14	.60	− 3.8	− 3.2	− 4.5
0 to 30	5.50	5.30	5.33	7.43	7.37	7.35	18.0	16.2	26.8	.37	.03	.38	−12.8	− 9.2	−14.7
30 to 60	6.00	5.51	5.77	7.59	7.57	7.52	41.3	34.5	51.9	.04	.00	.00	−31.8	−19.0	−34.1
60 to 90	5.00	4.34	4.60	7.55	7.57	7.52	37.8	26.9	42.1	.00	.00	.00	−28.6	−17.6	−30.3
90 to 120	3.63	3.50	2.70	7.48	7.47	7.49	24.1	18.6	21.3	.10	.00	.00	−17.9	−10.6	−16.5
120 to 150	3.04	2.90	2.77	7.45	7.43	7.38	16.7	12.2	14.3	.14	.06	.15	−12.9	− 7.4	− 9.5
150 to 180	2.97	2.67	2.53	7.41	7.40	7.36	13.5	9.8	10.7	.23	.04	.29	− 9.5	− 5.6	− 7.1
180 to 210	2.70	2.60	2.14	7.37	7.40	7.36	10.7	8.2	8.4	.27	.05	.38	− 7.5	− 5.2	− 5.1
210 to 240	2.50	2.27	1.93	7.38	7.40	7.33	8.4	6.4	6.6	.27	.10	.40	− 6.2	− 3.8	− 4.0
240 to 270	2.40	2.64	1.53	7.37	7.37	7.30	7.0	6.5	4.7	.29	.25	.35	− 5.6	− 3.9	− 2.7

* Urine parameter data were not available for experiments 3 and 4 (blood data in Table I) because of procedural errors in the analyses.

expressed as TA + NH_4^+ − HCO_3^- + RNH_3^+, are presented as equivalents of hydrogen ion excretion values in the formula, $UV_{H^+} = UV_{NH_4^+} + UV_{TA} + UV_{RNH_3^+} - UV_{HCO_3^-}$, of Nahas *et al*(2), which would therefore indicate a positive cumulative hydrogen ion excretion in the experimental animals during the period following THAM infusion. Since a true positive cumulative hydrogen ion excretion, under these experimental conditions, would necessarily effect acid-base changes in the blood of the experimental animals relative to the control animals in the direction opposite from that observed, it is evident that the latter formulation is in error.

The ammonium ion and the THAM ion (RNH_3^+) are chemically similar entities with similar properties in proton-transfer (acid-base) reactions. In titration measurements to normal blood pH, approximately 99% of the ammonium ion (pK = 9.3) and approximately 72% of the THAM ion (pK = 7.82) remain untitrated. On the other hand, the properties of these two ions with respect to biological systems are vastly dissimilar. The THAM ion is essentially non-metabolizable as well as relatively non-titratable, whereas the ammonium ion is very readily metabolizable and therefore capable of contributing its non-titratable excess proton quantitatively to the body buffers in consequence of its utilization in the biosynthesis of urea. The effects of ammonium ion excretion on physiological acid-base status might therefore be interpreted as owing to elimination of a metabolic precursor of hydrogen ion rather than of immediately available hydrogen ion, and the present data, which demonstrate that THAM ion excretion is not physiologically equivalent to ammonium ion excretion, are in accord with that interpretation.

Summary. The direction of change in acid-base status effected by urinary excretion of "net acid" plus THAM ion in dogs following THAM infusion was opposite to that predicted from the premise that THAM ion excretion has a physiological acid-base significance analogous to ammonium ion excretion.

ADDENDUM: The authors are indebted to the reviewer for bringing to their attention the following noteworthy points. a) Excreted RNH_3^+ may, to an appreciable extent, represent filtered RNH_2 which has, in effect, been titrated by the kidney. To this extent, urinary RNH_3^+ will be measured in the course of titration to blood pH and will be incorporated in the TA term of the "net acid" expression. b) Therefore, even if the UV_{H^+} ex-

pression of Nahas, *et al*(2) were otherwise acceptable, it would nevertheless need to be corrected by replacing the urinary RNH_3^+ term with a term representing solely the filtered RNH_3^+ ($UV_{H^+} = UV_{TA} + UV_{NH_4^+} - UV_{HCO_3^-} + C_{IN}P_{RNH_3^+}$). c) Values corresponding to filtered RNH_3^+ may be derived from total urinary THAM values by calculating the extent of ionization at blood pH. Hence, "UV_{H^+}" values corresponding to the "corrected" formulation may be calculated from the present experimental data.

Cumulative excretion values for filtered RNH_3^+ (calculated in the manner suggested by the reviewer) and for "H^+" calculated from the "corrected" formulation ("H^+" = "net acid" + filtered RNH_3^+) are shown in Fig. 4. Since the corrected values (Fig. 4) are not appreciably different from the uncorrected values shown in Fig. 1, it is evident that this correction eliminates only a relatively minor source of error. This result is in accord with the views already stated. Since RNH_3^+ cannot be metabolized, its presence in the organism can have no appreciable effect on acid-base status beyond the initial change brought about by its formation at the expense of hydrogen ion removed from blood buffers by interaction of the latter with infused RNH_2. The experimental data confirm that no further removal of hydrogen ion is effected as a consequence of RNH_3^+ excretion.

1. Elkinton, J. R., Huth, E. J., Webster, G. D., McCance, R. A., Am. J. Med., 1960, v29, 554.
2. Nahas, G. G., Reveillaud, R. J., Strauss, J., Schwartz, I., Verosky, M., Am. J. Physiol., 1963, v204, 113.
3. Simmons, D. H., Lewis, N., Ann. N. Y. Acad. Sci., 1961, v92, 559.
4. Jørgensen, K., Astrup, P., ibid., 1961, v92, 491.
5. Chapman, S. R., Smith, L. M., Simmons, D. H., J. Lab. Clin. Med., 1965, v66, 698.

Received December 17, 1965. P.S.E.B.M., 1966, v123.

Comparison of Intestinal Absorption and Esterification of 4-C^{14} Vitamin D_3 and 4-C^{14} Cholesterol in the Rat.* (31534)

NORMAN H. BELL†
Department of Medicine, Northwestern University Medical School, Chicago, Ill.

Cholesterol and other sterols are esterified during the process of intestinal absorption in rats(1-4). Further evidence is available that vitamin D also may undergo esterification (5). The purpose of the present study is to show that vitamin D is esterified during absorption. Further, the esters will be characterized on the basis of the degree of unsaturation of the fatty acid moiety.

Methods. Male Wistar rats weighing between 175 and 225 g were lightly anesthetized with ether. The thoracic duct was cannulated with a polyethylene catheter and exteriorized through a stab wound(1). The rats were placed in restraining cages and allowed free access to tap water and the Rockland "D free" diet (Teklad, Inc., Monmouth, Ill.). Twenty-four hours later they were given by intubation under light ether anesthesia 10^6 dpm 4-C^{14} cholesterol (145 μc/mg) (New England Nuclear Corp., Boston, Mass.) or 4-C^{14} vitamin D_3 (15 μc/mg) (N. V. Philips-Duphar, Weesp, Netherlands) dissolved in a drop of ethanol and diluted with 0.5 ml propylene glycol(5). The labeled sterols were purified immediately before use by chromatography on 20 × 20 cm glass plates covered with Silica Gel H (Brinkman Instruments, Westbury, N. Y.) in the solvent system methylene chloride. Lymph from the rats was collected in the next 24 hours and frozen until analyzed.

Aliquots of lymph were extracted by the method of Bligh and Dyer(6). The chloro-

* Supported in part by the Otho S. A. Sprague Foundation and in part by USPHS Grant AM 09016 from Nat. Inst. of Arthritis & Metab. Dis.

† Recipient of USPHS Career Development Award No. 1 K3 AM 25-441 from Nat. Inst. of Arthritis & Metab. Dis.