THE PHARMACOLOGY OF TRIS(HYDROXYMETHYL) AMINOMETHANE (THAM)

GABRIEL G. NAHAS

Department of Anesthesiology, College of Physicians and Surgeons, Columbia University, New York, N. Y.

TABLE OF CONTENTS

I. HISTORY

Enzyme chemists and biochemists have found very few acid salt buffer systems suitable for the regulation of pH in the physiological range of 6.5 to 7.5. Although some phenols, metaboric acid, barbituric acid, and the bicarbonate and biphosphate ions are effective in this range, their use is limited. Carbonate and phosphate, for example, precipitate calcium salts. Bicarbonate has a pK (6.1) in the lower part of the physiological range and can only be used in an open system. Barbituric acid has a low solubility and inhibits certain enzymes. Phenols and borates are too reactive in physiological media. In attempts to find more useful compounds, the nitrogen bases and their salts were investigated. Kirby and Neuberger (63) and Mertz and Owen (83) suggested imidazole, but although it is compatible with calcium, it reacts with a number of the enzyme systems. The ethanolamines, first used by Pardee (119) and Krebs (64) in manometric measurements, undergo auto-oxidation and are quite toxic *in vivo*.

In 1946 Gomori (42) suggested three amines for pH control in the range of 6.5 to 9.7: 2,4,6-trimethylpyridine (collidine), tris(hydroxymethyl)aminomethane or 2-amino-2-hydroxymethyl-1,3-propanediol, and the closely related 2-amino-2-methyl-1,3-propanediol. Tris(hydroxymethyl)aminomethane proved to be the most satisfactory of these compounds, and it has since been used extensively *in vitro* and, more recently, *in vivo*.

Since its introduction as a titrating agent in chemistry and biochemistry, this compound has been referred to by various names: 2-amino-2-hydroxymethyl-1,3-

propanediol, a designation used by Chemical Abstracts; 2-amino-2-hydroxymethylpropane-1,3-diol, a variation which the British chemists prefer; and tris(hydroxymethyl)aminomethane, the term used by the first commercial manufacturer (Commercial Solvents Corp.). Tromethane, Talatrol and Trizma are brand names. Tris, tris buffer, tris amino and trisamine are abbreviations frequently used by chemists, the two first being favored by biochemists. THAM is an abbreviation preferred by this author, because it corresponds to one of the chemical designations [Tris(Hydroxymethyl)Amino Methane].

II. PHYSICO-CHEMICAL PROPERTIES

THAM is an amino-alcohol derived from one of the nitroparaffins or nitroalkanes, CH_3NO_2. The condensation of a nitroparaffin with an aldehyde, yielding a nitro-alcohol $(CH_2OH)_3CNO_2$, was first described by Henry (47) in 1895. Amino-alcohols are formed from the nitro-alcohols by reduction. Tris(hydroxymethyl)aminomethane was first mentioned in 1897 by O. Piloty and O. Ruff (122). It was not until 1930, however, when Hass *et al.* (44) of Purdue University discovered the vapor phase reaction of alkanes with nitric acid, that preparations of nitro-alkanes became commercially feasible. In May 1940, nitro-alkanes and their derivatives were commercially available. Comprehensive reviews of the chemistry of nitro-alkanes were written by Gabriel in 1939 (37), Hass and Riley in 1943 (45), Degering in 1945 (27), and Levy and Rose in 1947 (67).

THAM is a white crystalline solid with a mw of 121. It is a weak base, *i.e.*, a proton acceptor, and in the presence of acids the following reaction takes place:

$$(CH_2OH)_3C - NH_2 + HA \rightleftharpoons (CH_2OH)_3C - NH_3^+ + A^-$$

In vivo the major source of the H^+ to be titrated is carbonic acid. The titration of free CO_2 and formation of HCO_3 by THAM are immediate, and there is no necessity, as was suggested by Burk (20), for the addition of carbonic anhydrase to speed the reaction. Unlike other amines, THAM can be easily prepared in a high state of purity and is now available in pure form or as a hydrochloride salt. Its physicochemical properties have been extensively studied by Bates (4, 5, 6), Fossum *et al.* (34), and Riddick (129) in descriptions of its use as a titrimetric standard.

The dissociation constant ($K_b \times 10^6$) of THAM in water is 1.202 at 25°C (6). It is very soluble in water but, unlike most amino-alkyl alcohols, has a low solubility in other liquids (36, 130), especially in oil (Table 1). Schulman and Montagne (143) studied the mechanism of the formation of microemulsions with amino-alkyl alcohols in terms of the structure of the interfacial mixed film produced by the adlineation of the hydroxyl groups when they adsorb as counter ions to the fatty acid molecules in the monolayer. If the amino-alkyl alcohol has one or two hydroxyl groups per molecule, as does, for example, 2-amino-2-methyl-1,3-propanediol, this adlineation permits intermolecular hydrogen bonding from which the interfacial lattice structure is formed. However, the

TABLE 1

Solubility of THAM; from Frederick (36) and Riddick (130)

Solvent	Solubility (T 25°) (mg/ml)	Reference
Water	550.0	36
Ethylene glycol	79.1	130
Methyl alcohol (anhydr.)	26.0	36
Methyl alcohol (anhydr.)	25.8	130
Ethyl alcohol (95%)	22.0	36
Ethyl alcohol (anhydr.)	14.6	130
Dimethylformamide	14.0	36
1-Pentanol (n-amyl alcohol)	2.6	130
Acetone	2.0	36
Ethyl acetate, abs.	0.5	130
Olive oil	0.4	36
Corn oil	0.3	130
Cyclohexane	0.1	130
Benzene	0.1	36
Chloroform	0.05	36
Carbon tetrachloride	<0.05	36

hydrogens of THAM are bonded intramolecularly and, like ammonia, it does not form microemulsions in respect to water-oil interfaces.

THAM is stable at room temperature for periods of as long as 12 years. It has a low hygroscopicity under the usual laboratory conditions, the critical humidity point being above 90% at 25°C. It may be dried by heating at 100°C but is unstable above 110°C. As a weak base, it absorbs minimal amounts of CO_2 from the air but not enough to alter significantly its properties as a hydrogen ion acceptor. The intramolecular hydrogen bonding of THAM, first described by Benesch and Benesch (8), may account for its high stability.

The stability of this compound has led to its extensive use as a titrating agent *in vitro*, in enzyme studies (77, 146), bacteriological media (116), protozoan nutrition (118), tissue culture (157, 158), yeast studies (135), and blood coagulation studies (151). Mahler (77) has, on the other hand, pointed out that THAM might interact in certain enzyme systems, *e.g.*, *in vitro* with carbonyl substrates, such as in the reaction of pyruvate with diphosphopyridine-nucleotide in the presence of lactic dehydrogenase and with substrates containing metals such as silver (8). Interactions with carbonyl groups may also occur with substrates that are aldehydes, ketones, keto-acids, and aldo- and keto-sugars. In all these cases, the thermodynamic activity of the substrates would be lowered and the structure altered, changing both the rate and extent of the reaction. Therefore, errors can be introduced in stoichiometric equilibria measurements, *e.g.*, in studies of the transformylation reaction.

The observations *in vitro* of Mahler indicate that in a wide variety of enzymatic reactions THAM cannot be considered chemically unreactive. *In vivo*, similar reactions could occur only inside cells. At pH 7.40 a significant fraction (30%) of the THAM is un-ionized and may penetrate cells. However, in view

of the large amounts of THAM which may be given without toxic or side-effects, it would seem that these interactions are not as extensive *in vivo* as one would expect from Mahler's studies.

The buffering capacity of THAM varies considerably with temperature. Buffer capacity is derived from "buffer value," defined by Van Slyke (163) as the differential ratio dB/dpH, where dB is the increment of base (in g equivalents) added to 1 liter of buffer solution, and dpH is the resultant increment in pH. When measurable increments are indicated by Δ,

$$\frac{dB}{dpH} = \frac{\Delta OH^-}{\Delta pH} = \frac{-\Delta H^+}{\Delta pH}.$$

Buffer capacity is measured by the slope of the titration curve with the pH plotted against base. It is dependent on the amount and composition of the buffer system and its temperature or, in other words, its actual pH. Therefore, all these factors must be clearly defined when THAM is used to titrate acid, *in vitro* or *in vivo*. At 25°C its pK_b is 8.08, and at 37°C it is 7.82 (57, 71). Its equivalence point at 25°C is 4.80, and at 37°C it is 5.89. A 0.3 M solution (36 g/liter), being very weakly ionized, is isosmolar with plasma and has a pH of 10.2 at body temperature.

III. METHODS OF ASSAY

Several bioassay methods for THAM have been reported. In the method described by Rosen (134), the basic compounds in the biological specimen are adsorbed by a resin (Dowex 50). The amines are released from the resin by the addition of acid and then oxidized with potassium dichromate. Concentrations of THAM in samples containing 0.2 to 10.0 mg/ml are measured with a colorimeter, and lower concentrations (0.02 to 0.2 mg/ml) with a spectrophotometer. This technique has been satisfactory for the determination of THAM concentrations in urine, but measurements in blood have been less consistent.

The method developed by Linn and Roberts (72) involves the quantitative oxidation of THAM to ammonia with alkaline periodate, using a Conway microdiffusion cell. After incubation overnight, the ammonia concentration is measured by a standard titration method. Clark (22) adapted the amino acid method of Folin, as modified by Frame *et al.* (35), for the measurement of THAM levels in blood. THAM can also be tagged with C^{14} and tracer amounts may then be used to determine concentrations in biological specimens (52).

IV. DISTRIBUTION AND EXCRETION

Studies by Ligou and Nahas (71) indicated that THAM is distributed into a volume larger than the extracellular space. It was, therefore, assumed that THAM penetrates into the cells. Linn and Roberts (72) infused a rabbit with 7300 mg of 0.3 M THAM (26.7 mmol/kg) over a period of 5 hours. At the end of the infusion 3540 mg of THAM had been recovered in the urine. Assuming that the remainder (3760 mg) was evenly distributed in body water (60% of body weight), the calculated concentration of 2.43 mg/ml of body water is in

good agreement with the measured blood concentration of 2.58 mg/ml. Clark (22) administered 300 mmol of THAM (21.5 mmol/kg) in 3 hours to a 14-kg dog the renal arteries of which had been ligated. At the end of 4 hours the concentration of THAM in the blood was 34 mEq/l, which corresponds to the calculated concentration in total body water (35.7 mEq/l).

Holmdahl and Nahas (53), using inulin and tracer amounts of C^{14}-labelled THAM, studied the volume distribution of THAM in nephrectomized cats and dogs. Arterial pH was maintained close to 7.40, at which pH 30% of the THAM is un-ionized. No C^{14} was detected in the expired air. After 15 minutes the volume of C^{14} space was significantly larger ($P < 0.02 > 0.01$) than the inulin space. Plasma radioactivity approached a plateau after 4 to 6 hours. Assuming minimal metabolic degradation and uniform distribution, the C^{14} was distributed into a volume corresponding to 55 ± 9% of body weight, which approximates the volume of total body water. Similar results were reported by Robin *et al.* (132) who also observed that the C^{14} decline in extracellular fluid followed a simple exponential form. Holmdahl and Nahas, however, with more frequent sampling, observed that the C^{14} decline in plasma was multiexponential in form. The C^{14} levels observed in cerebrospinal fluid and red blood cells indicated that THAM may not be distributed into all body compartments at the same rate. After 6 hours, the C^{14} concentration in the cerebrospinal fluid was only 7 to 15% of the plasma concentration. Carbon^{14} was detected in the red blood cells after 30 minutes to 1 hour and, after 5 hours, distribution between plasma and red blood cells approached equilibrium.

One might expect a compound of a molecular weight of 121, which is 30% un-ionized at pH 7.40 and 37°C, to equilibrate between the different body compartments at a rate faster than that observed. Omachi *et al.* (117) investigated volume changes of human erythrocytes in protein-free suspensions containing THAM (300 to 413 mOsmol/l). They observed a swelling of the red blood cells within 10 minutes, the rate of volume change being directly related to the un-ionized THAM concentration. *In vitro* studies performed by Holmdahl and Nahas (53) with red blood cells washed in saline showed volume changes within similar time limits.

The delay in equilibration *in vivo* could be better understood if THAM were protein-bound, as might be inferred from the studies of Rees *et al.* (126) and of Hamolsky and Stein (43). Dialysis studies with THAM in serum and serum albumin, using C^{14}-tagged material, were performed by Goldberg *et al.* (40). In 23 experiments, after equilibration there was no significant difference ($P < 0.01$) between the concentrations inside and outside the dialysis bag, indicating that THAM was not bound by the protein fraction of serum.

The rate of excretion of THAM by the kidney has been extensively studied. One hour after infusion of 0.3 M THAM, Nahas *et al.* (98) recovered 25% of the total amount administered in the urine. Brown *et al.* (18) administered 0.3 M THAM intravenously to healthy young men for 30 to 60 minutes. Twenty percent of the THAM was recovered in the urine after 1 hour, 60% in 24 hours, and up to 78% in 72 hours. Linn and Roberts (72) infused a rabbit with 0.3 M THAM

for a period of 5 hours followed by normal saline for 2 hours. At the midpoint of the infusion, 22% of the THAM was recovered in the urine, 48.5% was recovered at the end of the 5-hour infusion, and 66% after 6 and ⅔ hours. In additional studies on rabbits, they administered 80 ml of 0.3 M THAM/kg, titrated to pH 5.5 with hydrochloric acid. This infusion did not make the blood alkaline and, after 7 hours, 77% of the THAM was recovered, indicating that THAM is more rapidly excreted in its ionized form. Nahas and Reveillaud (108), using C^{14}-tagged material,* administered 0.3 M THAM (18 mmol/kg) i.v. for 1 hour to dogs maintained in apneic oxygenation. Under these conditions, THAM titrates endogenously produced CO_2 and the pH remains constant. At the midpoint of the infusion period 20% of the C^{14} was recovered in the urine, 30 to 35% at the end of infusion, 71% after 3 hours, and up to 93% after 3 days. Aliquots of the urine specimens collected in these experiments were chromatographed in a system of butanol saturated with ammonia, and were scanned to determine the nature of the C^{14} material excreted. The samples collected within 1 to 3 hours after THAM infusion showed R_f movement similar to that of the original C^{14}-tagged THAM. The 24-hour samples and the later ones had a different chromatographic pattern, indicating the presence of metabolites (24) which remain to be identified. Samiy (137) reported similar 1-hour recoveries of THAM but only 45% recovery after 18 hours.

These studies demonstrate that the excretion of THAM is not only a function of its total concentration in the plasma, but is also determined by the concentration of its ionized fraction. It will, therefore, be more rapidly excreted if kidney function is normal, when used to correct acidosis, since the blood pH will not become alkaline. The rapid excretion of THAM, as well as the delay in its equilibration in body water, may limit its penetration into intracellular spaces, especially when blood pH is normal. Under these conditions, if 70% of the material has been excreted during 3 hours (when equilibration is not yet complete), only a limited amount could have penetrated into cells.

V. TOXICITY

The acute toxicity of 0.3 M THAM injected intravenously (30 seconds) in mice was determined by Richards (128) and Roberts and Linn (131). The LD50 reported by Roberts and Linn was 16.6 mmol/kg, as compared to 11.5 reported by Richards. The LD50 of NaCl in isotonic solution administered to a control series was 36 mmol/kg. The addition of NaCl or dextrose to the THAM solution did not alter the LD50, but titration of THAM to pH 7.4 or 5.5 with hydrochloric acid seemed to increase the toxicity (LD50 12.9 and 8.7 mmol/kg, respectively). A dose of 16 mmol of 0.8 M THAM (administered i.v. in a single rapid injection) produced a decrease of the twitch response in the peroneal tibialis preparation of the cat (62). A dose of 4 mmol did not alter the response. The effect of THAM titrated to pH 7.40 was the same as with the unbuffered material.

* Kindly provided by Abbott Laboratories, North Chicago, Illinois.

The maximum subchronic, nonlethal dose of 0.3 M THAM for rabbits (131) is between 22.5 and 27 mmol/kg (administered i.v. daily over a period of 5 hours for 10 days). This amount is similar to the maximum subchronic nonlethal dose for dogs, as established by Richards (1.5 to 3 g/kg, administered i.v. at the rate of 0.5 ml/kg/min, 5 times weekly for 4 weeks). Roberts and Linn also observed that in contrast with acute toxicity studies, titration of THAM with HCl decreases subchronic toxicity.

A solution of 0.3 M THAM did not produce hemolysis of rabbit erythrocytes (131). Omachi *et al.* (117) studied human erythrocytes suspended in 300 mOsM THAM solutions. No hemolysis had occurred after 3 hours, but when the cells were allowed to stand overnight, hemolysis was observed in THAM solutions at pH 7.8 and 7.4 but not in those at pH 7.0 and 6.6, or in phosphate buffer at pH 6.5 to 7.7. By contrast, in isosmolar solutions at pH 7.4 of 2,4,6-trimethylpyridine, which has the same molecular weight as THAM, hemolysis was observed in 23 seconds. Another ring compound, imidazole, at pH 7.8, 7.4, 7.0, and 6.6, produced hemolysis in 35, 47, 104, and <600 seconds, respectively. These experiments are a further indication of the delayed intracellular penetration of THAM. Jørgensen and Astrup (57) noted a slight hemolysis when 1 volume of 0.3 M THAM (titrated to pH 7.40 with HCl) was added to 10 volumes of human whole blood and efficiently mixed. However, titrated THAM is hypertonic with reference to blood.

Roberts and Linn observed necrosis in rabbits around the site of infusion (marginal ear vein) after 0.3 M THAM administration (131). Animals which have received a lethal dose of THAM present severe necrosis at the point of infusion, but on histological examination the vital organs show lesions only due to secondary infection (128). Vasospasm of small veins has also been reported by many investigators (16, 120, 131). When THAM titrated to pH 7.40 is infused, the irritation is absent or markedly reduced, indicating that the alkalinity of the solution is the main cause of irritation. However, such titrated THAM, having lost more than two-thirds of its *in vivo* buffering capacity, has only a limited clinical application in the treatment of acidosis.

Although the intravenous infusion of THAM must be performed with caution, it is the only effective mode of administration. The intestinal mucosa preferentially allows the absorption of the un-ionized form of drugs or chemicals (49). THAM is present in the digestive tract primarily in its ionized form and, when given orally, acts mostly as an osmotic cathartic (personal observation). Only when administered by stomach tube in very large and concentrated doses (700 ml of a 10% solution) can it produce an effect on blood acid-base balance (16).

Berman *et al.* (12) reported that a dose of 3 to 5 mmol/kg administered to ten healthy volunteers for 30 to 60 minutes was well tolerated in all instances. In spite of a fall in ventilation, accompanied by CO_2 retention and a fall in O_2 saturation, no immediate or later reaction was noted. Brown *et al.* (17), in giving an equivalent dose to 3 healthy young males, made similar observations. Toxic manifestations were recorded after the administration of 8.8 mmol/kg for a

period of 60 minutes. These included transient but severe hypoglycemia, periodic breathing, retching, vomiting, and hypotension which persisted for 24 hours. Balagot *et al.* (2), however, have reported the administration of a similar amount (8 to 10 mmol/kg) over a period of 12 hours, to a patient with barbiturate poisoning. This patient was given electrolytes and glucose in addition to THAM. Except for local venospasm at the site of THAM infusion, there were no side effects observed and no assistance in ventilation was considered necessary.

Since THAM is excreted in the urine over a period of several days (see p. 452) the compound may accumulate in the body if large doses are given frequently. The amount that can be safely accumulated has not yet been determined.

VI. EFFECTS ON PHYSIOLOGICAL FUNCTIONS AND SYSTEMS

A. Acid-base balance

McFarland and Norris (75, 76) used THAM (2 to 20 mM) to stabilize pH and control CO_2 accumulation during transport of fish and were able to extend by 3 times the survival of such fish as compared to controls. They found that inorganic buffers, such as sodium monophosphate, sodium carbonate, and sodium bicarbonate, either caused the precipitation of calcium or magnesium salts in sea water or titrated outside the optimum pH range. The addition of THAM to a sealed compartment containing fish maintained pH within 0.1 unit for 25 hours, without affecting O_2 uptake. There was, however, CO_2 retention by the fish, indicated by a decrease in CO_2 production and an increase in R.Q.

Nahas (88) used THAM to correct acidosis *in vivo*. A solution of 0.3 M THAM in 0.3% NaCl was administered intravenously for 1 hour (1.1 ml/kg per min) to dogs with acute hypercapnic acidosis induced by apneic oxygenation (50). Arterial blood pH remained constant in the presence of a marked CO_2 retention, which was primarily in the form of HCO_3^-. The untoward effects usually associated with severe hypercapnia of this type (intracranial hypertension, bradycardia, hypertension followed by circulatory collapse, anuria, elevated plasma catecholamines) did not occur (56, 97, 110). Eighteen to 28% of the estimated total amount of CO_2 produced during apnea was excreted by the kidney (98). There did develop, however, a marked hypoglycemia which disappeared 30 to 60 minutes after the end of the infusion. These experiments, which were later confirmed by Millar *et al.* (85), by Baratz (3), and by Manfredi *et al.* (78, 79), led to the following conclusion: "The dog will tolerate well a plasma concentration of CO_2 over twice its normal level when its two fractions, HCO_3^- and H_2CO_3, are in suitable proportion to maintain the biological neutrality of the internal environment" (88).

No precision was given at first to the extent of the titration by THAM of CO_2 in the different body compartments. In subsequent experiments Nahas *et al.* (94) infused Na_2CO_3 during similar periods of apneic oxygenation, and although arterial blood pH remained normal, they observed marked cardiovascular disturbances. Titration of the CO_2 in the blood alone did not prevent some of the untoward effects of CO_2 accumulation. Measurements made with tracer amounts of C^{14}-tagged THAM (109) during apneic oxygenation indicated that after 1 hour,

THAM was distributed in 27 to 37 % of body weight, a volume which is larger than the extracellular volume as defined by inulin space. It is, therefore, probable that THAM acts as a proton-acceptor inside cells and titrates intracellular CO_2.

Robin *et al.* (132) used the 5,5-dimethyl-2,4-oxazolidinedione (DMO) technique to measure "intracellular" pH and concluded that THAM exerts a buffering action inside cells. Sixty minutes after the intravenous infusion of 150 mmol of THAM to dogs there was a significant rise in intracellular pH (from 7.08 to 7.27) accompanied by a marked increase in "apparent bicarbonate" concentration of intracellular fluid. Intracellular pH rose somewhat more than extracellular pH, as might be expected because of the lower buffering capacity of the tissues. At pH 7.40 THAM is 70 % ionized in normothermic blood and 1.3 mole is required to titrate each mole of CO_2 absorbed by the blood, whereas in intracellular fluid, at pH 6.9 and with 90 % of the THAM in its ionized form, 1.1 mole is required. However, as suggested by Fenn (33): "If un-ionized THAM is equally distributed in all body water, the cells would be overneutralized and an abnormally large fraction of the CO_2 absorbed by the whole body would be in the cells. As a result K^+ might move out of the cells to restore the normal Donnan ratio." This does seem to occur when cases of severe acidosis are rapidly and massively corrected by addition of THAM (32).

Ligou and Nahas (70, 71) used THAM to titrate "addition acidosis," produced by infusion of lactic acid, and demonstrated that it is as good a buffer in metabolic as in respiratory acidosis. Peirce administered THAM to correct severe mixed acidosis in dogs maintained on cardiac bypass, with a low flow of 30 ml/kg per min (121). It was used by Benichoux *et al.* (9a) to correct the acidosis produced by temporary occlusion of the thoracic portion of the inferior vena cava. Studies have been made (46, 82) on the effects of controlling pH during the course of hemorrhagic shock, which is accompanied by a severe metabolic acidosis. In order to avoid depression of ventilation, a solution containing 75 mmol of $NaHCO_3$ and 150 mmol of THAM was used. The correction of pH alone did not alter the number of survivals. However, when maintenance of a normal pH was combined with an increased O_2 supply in the experiments of Manger *et al.* (82, 104), a significant increase in the number of survivals was observed.

Jørgensen and Astrup (57) studied the effect of THAM on blood buffering capacity and reported that the addition of 50 mmol/l or less to plasma did not change the pK value (6.10) of carbonic acid. The behavior of THAM in the blood could be predicted from its properties as a weak base without chemical side-reactions and from the properties of blood.

There has been much discussion of the possible role played by free CO_2 accumulation in the toxicity observed during exposure to high oxygen tensions. Studies have been made of the effect of THAM administration on O_2 toxicity in mice (111, 140, 141). Thirty minutes after a single intraperitoneal injection of 1 ml of 0.3 M THAM, the mice were exposed to 30, 42, and 55 *psi* of oxygen. There was a significant delay in the appearance of convulsions. This delay did not occur after a single injection of 0.3 M $NaHCO_3$. However, 3 injections of 0.3 M THAM

or 0.3 M $NaHCO_3$ administered over a 6-hour period were equally effective. Oxygen toxicity, therefore, may be associated with acid-base imbalance which can, to some extent, be alleviated by the administration of buffers. The exact nature of the acid-base imbalance and its correction is still to be determined. Bean (7) made similar observations in rats, exposed to 80 *psi* of O_2 8 to 10 minutes after the injection of a 10% solution of THAM (1.5 g/kg) in isotonic saline solution. THAM postponed the onset of seizures and decreased their incidence and severity. Lung damage was either absent or much less severe than in control animals, and mortality rate was lower (14% instead of 38%). Bean suggested that such highly significant ($P < 0.01$) results should redirect attention toward increased tissue pCO_2 and tissue (H^+) concentration as possible contributors to the toxic reaction to high oxygen pressures.

THAM has been used clinically in the treatment of severe respiratory or metabolic acidosis. Brinkman *et al.* (14, 16), who reported its first clinical use, administered by gastric tube as much as 2 g/kg in a 10% solution. This method of administration corrected arterial pH, but was accompanied by diarrhea. They also gave THAM orally and intravenously to emphysematous patients with severe CO_2 retention. This therapy, however, was accompanied by a depression of ventilation and, in one case, by apnea. Similar observations were reported by Luchsinger (74), Manfredi (80, 81), Conant and Hughes (23), Sieker (147), and Swanson (156). In the subsequent use of the buffer by these investigators, therefore, patients were mechanically ventilated to compensate for THAM's depressant effect on ventilation (95). Luchsinger (73) has demonstrated that this effect precludes the administration of THAM to patients with chronic respiratory acidosis. For the same reason THAM cannot be used to prevent CO_2 toxicity in a confined environment (142).

Kaplan *et al.* (58) used THAM to treat mixed and respiratory acidosis in 6 children, 5 of whom were considered to be in a moribund state in spite of conventional therapy. A 0.3 M THAM solution, containing 30 mEq of NaCl and 5 mEq of KCl, was administered at rates of 0.4 to 26.9 ml/kg per hour for periods of 0.4 to 52.8 hours. The acidotic trend was reversed with a dose of 5 ml/kg per hour; the total administered in 24 hours did not exceed 300 mg/kg. Ventilation was controlled in 5 of these patients. In all cases the acidosis was corrected, and 5 of the 6 patients survived. S. A. Kaplan (59) has commented further on the therapeutic indications of THAM in pediatrics.

THAM administration was used by Peirce (121) to correct the metabolic acidosis which develops in the course of intracardiac surgery performed with hypothermia, and by Clark (22) to correct the metabolic acidosis which develops following heart surgery. Rees *et al.* (126) and Samiy *et al.* (139) treated diabetic acidosis with THAM and recommended a dose of 100 mEq to be administered with 70 mEq of NaCl over a period of several hours.

The technique of apneic oxygenation was first applied during bronchoscopy by Holmdahl (50). In the course of 10 bronchoscopic procedures he administered 0.33 M THAM intravenously (0.53 ml/kg per min) during 6 minutes of apneic oxygenation (51). Arterial blood pH remained constant during THAM

administration, the amount infused being only slightly higher than predicted on the basis of the stoichiometric reaction between THAM and CO_2. This administration was well tolerated by all the subjects, none of whom complained of the headaches which occasionally occur after periods of apneic oxygenation. When bronchoscopy with apneic oxygenation is performed, therefore, a sudden rise in blood pressure or intracranial pressure can be prevented by the administration of THAM (51).

B. Renal function

Early experiments had demonstrated that animals receiving THAM during "apneic oxygenation" have a profuse water and electrolyte diuresis accompanied by a fall in plasma sodium and chloride levels but no change in potassium (98). The rapid excretion of THAM observed during the diuresis suggested that this compound acts as an osmotic diuretic.

In studies on the renal effects of THAM in the dog, Samiy *et al.* (136, 137) infused solutions of 0.3 M or 0.6 M THAM and determined bicarbonate and electrolyte clearances. There was a marked increase in blood and urinary pH and a 10-fold increase in the rate of urine flow. The excretion of Na^+ and Cl^- increased to 10 to 15% of the filtered load, paralleling the increase in the rate of urine flow as well as the molar concentration of the THAM infused. Ninety to more than 100% of the filtered K^+ and 50 to 60% of the filtered HCO_3^- were recovered in the urine. These studies also demonstrated that THAM behaves as an osmotic diuretic but the disproportionate increase in K^+ and HCO^-_3 excretion, as compared to Na^+ and Cl^-, indicated that it might also have a direct action on the kidney tubule. Samiy suggested, therefore, that THAM penetrates into the cells and interferes with the (H^+)–(Na^+) exchange mechanism. Similar conclusions were reached by Portwood (123).

Nahas *et al.* (109) studied acid excretion in dogs during THAM administration under conditions of constant CO_2 load and normal blood pH. A 0.3 M THAM solution, containing tracer amounts of C^{14}-tagged THAM, was administered during 1 hour of apneic oxygenation. The observations of Samiy and Portwood were extended and confirmed. The osmotic diuretic activity of THAM was demonstrated by the following findings: urine osmolality was close to plasma osmolality; the diuresis and Na^+ excretion rate were parallel; the rapid elimination of THAM (75% or more after 8 hours) was comparable to that of other osmotic diuretics. The K^+ elimination, as in Samiy's study, was greater than might be expected and, in 5 of 10 cases was more than 100% of the calculated filtered load. There was an increase in the filtered load of bicarbonate but the tubular reabsorption of bicarbonate increased only during the first 20 minutes of THAM administration and then remained constant. THAM excretion was also greater than the calculated filtered load, indicating that THAM might penetrate into the tubular cells.

Rector *et al.* (125) have shown that an increase in HCO_3^- reabsorption accompanies a rise in plasma pCO_2. Samiy *et al.* (137) observed a decrease in bicarbonate reabsorption during THAM administration when plasma pCO_2 was

constant and pointed out that this decrease could be due to an interference with the (H^+)-(Na^+) exchange mechanism within the tubular cell. The absence of an increase in bicarbonate reabsorption in the experiments of Nahas *et al.* during most of the infusion period, in spite of a rising pCO_2, is further evidence of this mechanism.

The administration of THAM during hypercapnic acidosis was accompanied by a marked increase in H^+ excretion, as calculated using the following formula:

$$HV_{H^+} = UV_{NH_4^+} + UV_{TA} + UV_{R\cdot NH_3^+} - UV_{HCO_3^-}$$

where $R\cdot NH_3^+$ = ionized THAM. An early assumption (98) that ionized THAM was equal to the cationic deficit calculated in the urine, was confirmed by measurements with C^{14}-tagged THAM. The cationic deficit was less than 10% of the ionized THAM excreted (164). In the body at pH 7.40, 70% of THAM reacts with H_2CO_3 as follows:

$$(CH_2OH)_3C-NH_2 + CO_2 + H_2O \rightleftharpoons (CH_2OH)_3C-NH_3^+ + HCO_3^-.$$

During apneic oxygenation and THAM administration, each amine group of the THAM excreted carries with it a H^+, the kidney acting as an outlet for the H^+ fixed in body fluids by THAM. Simmons and Lewis (65, 66, 148, 149, 150) superimposed, by hypoventilation, a respiratory acidosis on the metabolic alkalosis produced by an infusion of 0.27 M THAM or 0.15 M sodium bicarbonate. They measured titratable acidity of the urine, added these results to the NH_4^+ excretion, and concluded that: "the decrease in acid excretion was less following bicarbonate than following THAM." Simmons, however, did not measure nor take into account the excretion of ionized THAM.

In 7 of 10 animals studied by Nahas *et al.*, the amount of bicarbonate excreted was smaller than the ionized THAM excreted, ionic equilibrium being satisfied by chloride ions. When excreted with chloride, therefore, cationic THAM may limit HCO_3^- excretion.

Berman *et al.* (13) made renal studies in six normal adults during and following the intravenous infusion of 0.3 M THAM (3 to 5 mmol/kg) for 30 to 60 minutes. The infusion also contained 30 mEq of NaCl + 5 mEq of KCl/l. Endogenous creatinine clearance remained unchanged. The elimination of Na^+, K^+, HCO_3^-, and Cl^- markedly increased, the Cl^- excretion exceeding the combined excretion of Na^+ and K^+. Both HCO_3^- reabsorption and excretion were increased. The recovery of THAM in the urine varied directly with the infusion rate. Similar results were reported by Tarail *et al.* (161).

THAM administration is accompanied by a marked diuresis of alkaline urine and, since an alkaline urine enhances the excretion of weak acids, it was postulated that THAM would increase the renal secretion of salicylic acid. Strauss and Nahas (152) administered sodium salicylate (100 to 200 mg) and THAM (4 to 6 mmol/kg) intravenously to 4 dogs. Fifteen minutes following THAM infusion, salicylate excretion in the urine had increased 4-fold, and 75 minutes after, blood levels had fallen from 33 to 17 mg per 100 ml. Clark (21) reported the first successful use of THAM in the treatment of a child with salicylate intoxication.

Israels (55) administered THAM (0.3 M solution, 500 mg/kg) for 1 hour to 4 children with salicylism. Three hours after the infusion, plasma salicylate levels had fallen 35 to 67 %, and 475 to 1817 mg of salicylate had been excreted. Balagot *et al.* (2) infused 72 g of THAM (0.3 M) over a period of 12 hours to a patient with barbiturate intoxication. Seven hundred and seventy-two mg of barbiturate were recovered in the urine, and plasma levels fell from 100 μg to 61 μg/ml. Reveillaud (127) reported a similar case of barbiturate intoxication in a hypertensive patient successfully treated with THAM administration.

Vick *et al.* (165) administered THAM in a hypertonic solution (4 % in 5 % dextrose, equivalent to 0.6 M) to dogs with endotoxin shock and hypotension, and observed a marked diuresis. Other investigators (38, 139, 144) have reported that an intravenous infusion of 0.6 M THAM produced a marked diuresis in dogs with hypovolemic hemorrhagic shock (mean blood pressure of 40 mm Hg). Solutions of 0.6 M NaCl or 4.5 M urea did not prevent the anuria of hypovolemic shock.

C. Electrolyte balance

The administration of 18 mmol/kg of 0.3 M THAM to dogs was accompanied by a fall in Na^+ and Cl^- plasma levels, while K^+ did not change (see p. 457). The plasma osmolal concentration, however, remained constant, because of the increase in HCO_3^- and the addition of ionized THAM, which compensated for the decrease in the other electrolytes. The Na^+ and Cl^- excretions were considerably greater than the decrease in plasma levels, indicating a significant movement of these electrolytes from the tissues into extracellular spaces. The plasma K^+ level remained constant, in spite of a high elimination, indicating that the shift of K^+ out of the cells was especially marked. A similar shift was observed by Rothstein (135) and Packer *et al.* (118) in studies on unicellular organisms.

These marked electrolyte shifts were not observed by Berman *et al.* or by Tarail *et al.* (see p. 458), but during their studies the amounts of THAM administered varied from 3 to 8 mmol/kg and electrolytes were added to the infusate to compensate partially for urinary loss. Nahas *et al.* (98) also reported the addition of 30 mEq of NaCl and 5 mEq of KCl/l to the buffer solution to compensate for loss of electrolytes. Plasma levels, therefore, did not change and equilibrium between extra- and intracellular spaces was maintained. Berman, however, did observe a rise of 0.2 to 0.8 mEq K/l in serum in 3 normal subjects. Tarail and Bennett (160) reported a 20 % increase in plasma K^+ following the infusion of 0.3 to 0.6 M THAM (10 to 12 mmol/kg) for 17 minutes to normal unanesthetized dogs. Potassium returned rapidly to preinfusion levels. A 50 % increase in K^+ level was observed in diabetic pancreatectomized dogs receiving a similar infusion of THAM.

The K^+ shift may be enhanced by a too rapid correction of acidosis in which case there is a transfer of K^+ from the cells into the extracellular fluids, as shown by Epstein *et al.* (32). Epstein gave an intravenous infusion of 2 M THAM at the rate of 1 to 2 ml/kg per min for 5 minutes to dogs which had been breathing

high concentrations of CO_2 for 4 hours. The plasma K^+ concentration, which rose markedly during hypercapnia, continued to increase after the infusion in spite of the considerable plasma dilution and a marked fall in plasma Na^+. This agrees with the observations made by Fenn (see p. 455). Fenn's observations were confirmed further by the studies of Rosano *et al.* (133) who showed that the rate of diffusion of NaCl and KCl through lipid membrane monolayers can be accelerated or decelerated by addition of OH^- or H^+ ions, respectively.

Samiy *et al.* (138) observed hyperkalemia following THAM administration in 2 of 4 patients with metabolic acidosis due to renal disorders. In one case, normal serum K levels were restored only after hemodialysis. Samiy also reported marked hyperkalemia after the infusion of large amounts of THAM to dogs with bilateral ligation of the ureters. It is, therefore, extremely important to emphasize that THAM treatment should be cautiously prescribed for patients with chronically impaired kidney function. In such cases its administration should be carried out slowly over a number of hours and should be accompanied by constant monitoring of urine flow and serum K^+ levels.

D. Ventilation

Nahas and Lumpkin (102) studied the effects of THAM on the ventilation of resting non-narcotized dogs breathing 5% CO_2 in air. After 10 minutes, $\dot{V}_E$ (minute ventilation) fell to or below control levels, while arterial blood pH and pCO_2 increased. The fall in $\dot{V}_E$ occurred at the expense of tidal volume, ventilation rate being the same as during the control periods of CO_2 breathing. It was, therefore, suggested that: "during THAM administration, ventilation varies with arterial blood pH changes and independently of $PaCO_2$ (arterial CO_2 tension) changes." This conclusion was not confirmed by subsequent observations on trained, awake dogs having permanent tracheal fistulae and implanted arterial catheters (112). The animals breathed from a spirometer containing 100% O_2. The administration of 0.3 M THAM (0.5 ml/kg per min) produced a 26 to 33% fall in $\dot{V}_E$ which was apparent 1 minute after the start of the infusion. The pH of arterial blood rose and $PaCO_2$, after an initial drop, also rose. Ten minutes after the end of the infusion, the $\dot{V}_E$ had returned to control levels while pH remained elevated. Equivalent amounts of $NaHCO_3$, however, produced an increase in $\dot{V}_E$ with similar changes in pH and $PaCO_2$. THAM, titrated with HCl to pH 7.0 or administered with equivalent amounts of $NaHCO_3$, did not change or only slightly depressed $\dot{V}_E$.

Katz *et al.* (60, 61) and Ngai *et al.* (113, 114) studied the effects of THAM on respiration, arterial blood pH, pCO_2, and O_2 saturation in decerebrate, pontile, and medullary cats. A solution of 0.8 M THAM, infused at a rate of approximately 0.3 mmol/kg per min (total dose of 5 mmol/kg), produced hypopnea or apnea. Arterial pH rose and the pCO_2, after an initial drop, also increased.

In studies on ventilation in normal, healthy adults, O'Connor *et al.* (115) administered 0.3 M THAM (4 ml/min) for 30 to 60 minutes to 4 fasting and supine volunteers. The THAM solution also contained 5 mEq KCl/l and 30 mEq NaCl/l. There was a 30% decrease in $\dot{V}_E$ together with a 33% decrease in

CO_2 output, with ventilatory rate remaining constant. In every case, the respiratory exchange ratio dropped to values between 0.49 and 0.73. Arterial blood pH increased an average of 0.05 unit, $PaCO_2$ rose, and oxygen saturation fell below 95%.

Brown *et al.* (17) gave THAM intravenously to 4 volunteers during CO_2 retention induced by the administration of CO_2 in air. The increase in $\dot{V}_E$ induced by CO_2 breathing was greatly reduced or abolished during THAM infusion because of a decrease in tidal volume. Carbon dioxide output dropped 67 to 123 cc/min, 12 to 24% of the retained CO_2 being excreted during the infusion. Arterial blood pH increased by 0.08 to 0.18 unit but $PaCO_2$ tension, after an initial transitory decrease of 3 mm Hg, showed little change. When CO_2 breathing was continued after the end of THAM infusion, $\dot{V}_E$ again increased, but pH remained elevated, and arterial and alveolar CO_2 tensions did not change. Oxygen uptake was essentially constant throughout, except during instances of marked hypoventilation (largest doses of THAM), when arterial hypoxemia was observed. These observations in man, as well as those of Nahas *et al.* in the dog, indicate that the depression in $\dot{V}_E$ that occurs with THAM administration cannot be accounted for by changes in either arterial pH or pCO_2. It is possible that the un-ionized fraction of THAM, which penetrates into cells, may affect receptor sites that are also sensitive to changes in pCO_2. Intracellular changes in pCO_2 or THAM concentration will, in turn, alter the H^+ or HCO_3^- concentration. These intracellular acid-base changes will be greater than those in the plasma, which has a much larger buffering capacity. One could, therefore, postulate that the changes in $\dot{V}_E$ observed during THAM infusion were dependent upon unequal alterations in extra- and intracellular $[H^+]$ or $[HCO_3^-]$ concentrations. Simultaneous measurements of $[H^+]$ in both compartments would be required to confirm this hypothesis.

E. Glycemia

The hypoglycemic activity of THAM was first reported by Tarail *et al.* (161) after studies performed with normal volunteers. The fall in glucose concentration, accompanied by a fall in phosphate, was significant only when doses in excess of 500 mg/kg (4 mmol/kg) were administered within 1 hour. In experiments on anesthetized dogs (160) after a total dose of 40 to 45 mmol/kg of 0.3 M THAM (infused over 3 to 4 hours), blood sugar levels fell from 100 to 122 down to 10 to 43 mg per 100 ml, and serum inorganic phosphate fell to less than 50% of control values. The fall in phosphate probably represented a net transfer from extracellular fluid to cells, since phosphate excretion decreased. Blood pH rose 0.24 to 0.30 unit and plasma CO_2 content increased 4 to 9 mM/l. There was a significant decrease in both serum sodium and chloride. Tarail *et al.* observed also that glycosuria did not develop in man or in dogs during THAM administration. Tarail and Bennett (160) administered THAM (10 to 12 mmol/kg) intravenously to trained, awake dogs for 17 minutes. Twenty minutes after the end of infusion there was a 30 to 59% drop in the plasma glucose concentration which returned toward control values 1 to 2 hours later. An infusion of

$NaHCO_3$ produced no change in plasma glucose levels despite increases in pH and CO_2 similar to those occurring with THAM. Blood pH rose 0.21 to 0.28 unit. The administration of 4 mmol of THAM/kg produced a slight fall (13%) in plasma glucose in only 1 of 4 normal dogs. An infusion for 17 minutes of 10 to 14 mmol of titrated THAM (pH 6.1)/kg produced a 21% fall in glucose, and recovery to normal levels occurred within 20 minutes.

Nahas *et al.* (93, 100) studied the effect of blood pH on the hypoglycemic activity of THAM. A 0.3 M THAM solution (18 mmol/kg), titrated to pH 7.40 with HCl, was administered to dogs for 1 hour and blood pH was maintained at various levels by mechanical ventilation. When the pH was maintained around 7.40 during THAM administration, blood sugar fell from 110 to 60 mg per 100 ml. When pH was maintained at 7.00 throughout the infusion of titrated THAM, no hypoglycemia was observed. When blood pH was further reduced to 6.80, the infusion of THAM was accompanied by an increase in blood sugar, from 110 to 180 mg per 100 ml. It seems likely, therefore, that the hypoglycemic activity of THAM is related to its un-ionized fraction ($R—NH_2$), *i.e.*, the fraction which penetrates into intracellular compartments.

Dos and Nahas (29, 91) administered 0.3 M THAM (12 to 20 mmol/kg) intravenously for 40 to 60 minutes to unanesthetized, restrained, pancreatectomized dogs. The animals had very high control blood glucose levels ranging from 301 to 568 mg per 100 ml. At the end of THAM administration, blood sugar levels were only slightly depressed (10 to 15%), while urinary glucose decreased from 8 to 10 g per 100 ml down to 0.3 to 0.5 g per 100 ml, with a 3- to 4-fold increase in diuresis. Similar observations were made on the phlorizinized dog (92, 154). The decrease in urinary glucose in the pancreatectomized animal could be accounted for by a decrease in glomerular filtration rate (GFR) as shown by Strauss *et al.* (153, 155). Since less glucose was filtered at the glomeruli, the glucose fell below its threshold for reabsorption. This decrease in GFR in the pancreatectomized dog contrasts with the stable GFR maintained in the normal animal during THAM administration. A second series of experiments demonstrated that a similar amount of THAM administered to dogs with phlorizin-induced glycosuria does not decrease glomerular filtration rate or glucose clearance (154, 155). In addition, in some cases there was an increase in tubular reabsorption of glucose, suggesting that THAM may counteract phlorizin's inhibitory effect on the tubular reabsorption of glucose.

Bennett and Tarail (10), after preliminary tests with normal animals, gave a single rapid infusion of THAM to pancreatectomized dogs deprived of food and insulin for 18 to 24 hours. There was no significant drop in glucose levels, but plasma K^+ increased by as much as 55%. However, an infusion of THAM a few hours after pancreatectomy produced a hypoglycemic response similar to that occurring in the normal dog. These investigators also directly perfused the pancreas of normal anesthetized dogs with a THAM solution titrated to pH 7.40 (0.02 to 0.06 mmol/kg). Plasma glucose levels fell 10 mg per 100 ml and remained at this level for 1½ to 2 hours, a result similar to that observed following the injection of 5% glucose. Seltzer and Smith (145), on the other hand, observed

no change in insulin activity in pancreatic duodenal vein blood following THAM administration to phlorizinized dogs. Bennett and Tarail also administered 9 mmol of THAM i.v. to normal dogs over a period of 30 minutes and observed a hypoglycemic response similar to that occurring after administration of 0.5 g glucose/kg per hour (the rate reported to be near the maximal intestinal absorption rate). The hypoglycemic response with THAM, as with glucose, appeared in 20 minutes, reached a maximum at 40 minutes, and was compensated within 2 hours. The administration of K^+ with the THAM increased the severity of the hypoglycemia. Bennett and Tarail concluded that while THAM may stimulate insulin secretion, it may also facilitate the activity of insulin itself. Therefore, the relationships between the effects of THAM on K^+ exchange, glucose metabolism, and insulin activity should be further investigated. Rees *et al.* (126), after paper electrophoresis studies *in vitro*, suggested that tris maleate buffer releases insulin normally bound to globulins in other systems at pH 8.6. However, the extent of this interaction of THAM with protein-bound insulin has not been demonstrated *in vivo*. The doses of THAM used by Bennett and Tarail decreased arterial O_2 saturation in the blood and possibly O_2 delivery to the tissues. This could increase glucose metabolism as demonstrated by Randle and Smith (124), who studied glucose uptake in the isolated rat diaphragm. However, Randle and Smith also demonstrated that in THAM medium buffered to pH 7.40, glucose uptake by muscle was not stimulated by anaerobiosis, as it was in the presence of $NaHCO_3$.

F. Thyroid hormones

Hamolsky and Stein (43) demonstrated that the binding of thyroxin (T_4) and of triiodothyronine (T_3) to plasma proteins was markedly increased in the presence of THAM. THAM also significantly increased the transfer of I^{131}-T_4 from human serum albumin across a dialysis membrane, into the surrounding plasma medium, indicating that the binding capacity of plasma had been increased. As a corollary, these authors have shown that *in vivo* pH, independently of pCO_2, plays a major role in the binding of T_3 and T_4 in dog blood, the uptake by red cells increasing at acid pH (less plasma protein binding) and decreasing at alkaline pH (more plasma protein binding). The marked alterations in pH which can be produced by THAM, in the presence of a more or less stable pCO_2, could, therefore, markedly change thyroid hormone transport and utilization.

G. Sympatho-adrenal systems

Ligou and Nahas (69) and Nahas *et al.* (89, 99, 101) studied the effect of pH changes on O_2 uptake and plasma catecholamine levels in the dog. During hypercapnic acidosis, induced by apneic oxygenation, there was an increase in plasma catecholamines in the presence of a constant oxygen uptake (Q_{O_2}) and a rise in mean blood pressure. When pH was maintained constant or alkaline by infusion of THAM, Q_{O_2} remained constant and, in spite of a 2-fold increase in total plasma CO_2, plasma catecholamines and blood pressure did not change. When blood pH was rapidly shifted from 6.99 to 7.52, Q_{O_2} increased by 39% and plasma catechol-

amines rapidly returned to normal levels. The infusion of epinephrine, norepinephrine or isoproterenol (1 μg/kg per min) also produced a significant increase in Q_{O_2} and blood pressure when arterial blood pH was maintained normal or alkaline with THAM. When arterial blood pH was maintained between 6.97 and 7.28 the same dose of epinephrine failed to change Q_{O_2} or blood pressure significantly.

The addition of THAM, therefore, suppresses the sympatho-adrenal stimulation observed during hypercapnic acidosis and, by restoring blood pH to normal, permits the catecholamines to exert their maximal activity. Nahas *et al.* (94), using Na_2CO_3, observed that maintenance of a normal blood pH alone does not prevent the stimulation of the sympatho-adrenal system in the presence of an elevation of $PaCO_2$ similar to that observed during administration of THAM. They concluded that THAM might act, in part, on the sympatho-adrenal system as an intracellular [H^+] acceptor.

Mittelman *et al.* (86, 87) measured adrenal blood flow and the adrenal vein concentration of compound F during hypercapnic acidosis and after its correction with THAM. During acidosis there was a marked rise in compound F concentration in the adrenal vein and a fall in adrenal vein pH to 6.8 to 6.9. When the acidosis was corrected with THAM, compound F in the adrenal blood rapidly returned to normal levels. It remains to be determined whether these alterations in adrenal cortical activity were mediated through the sympathetic system and an increased production of catecholamines, or through pH changes only, or through another mechanism. However, these observations do indicate that the correction of hypercapnic acidosis with THAM rapidly restores adrenocortical activity to normal.

In studies of "transfusion acidosis" by Nahas *et al.* (103, 104, 105), dogs were bled (48 to 50 ml/kg) and reinfused with blood collected in standard acid-citrate dextrose (ACD) solution. Fifteen of 17 animals suffered cardiac arrest during transfusion. Similar observations were reported by Baue *et al.* (6a), who perfused the isolated dog heart with ACD blood and produced cardiac arrest. The only marked electrolyte change observed was an expected increase in plasma Na^+. The major cause of death was concluded to be the acidity of the blood (pH 6.4 to 6.6) due to the pH of the ACD (103, 121), since death also occurred when dogs were transfused with heparinized blood acidified to pH 6.5 with HCl (2.5 mEq/100 ml). When THAM was added to the transfusion blood (2 to 3 mmol/100 ml) and pH restored to normal, 14 of 16 dogs survived. It was also observed during these transfusion studies that plasma catecholamine levels which had been elevated during the hypotension caused by hemorrhage were not restored after transfusion with ACD blood. However, the highest catecholamine concentration after transfusion was observed in dogs with the lowest pH, and the response of the sympatho-adrenal system may have been due to acidosis as well as hypotension. The restoration of both blood volume and blood pressure and maintenance of a normal pH with THAM was accompanied by a return of the catecholamines toward normal levels. These results suggest that following hemorrhage and blood replacement, markedly increased levels of endogenous

catecholamines will not sustain cardiovascular function if the pH is too low. Nahas *et al.* (105) and Baue *et al.* (6a) concluded that following cardiac arrest and massive blood replacement in surgery, and after exchange transfusion of the newborn with ACD bank blood, buffering of the blood and addition of Ca^{++} before transfusion should be considered.

H. Cardiovascular systems

Darby, Aldinger and Thrower (1, 25, 26, 162) studied the effect of THAM administration on ventricular contractile force in the dog following metabolic and hypercapnic acidosis. Metabolic acidosis was produced by total body perfusion with low flow rates during cardiac bypass and by intravenous infusion of lactic acid. The acidosis was accompanied by a decrease in ventricular contractile force and a reduction in the inotropic response to injections of levarterenol. THAM administration produced a rise in arterial pH and an increase in ventricular contractile force. The response to injections of levarterenol was restored or augmented, resulting in a marked increase in cardiac output. THAM was more effective than $NaHCO_3$ or Na lactate in restoring the myocardial response to catecholamines. Overcorrection of the acidosis seemed to depress sympathoadrenal tone, elicit cerebral vasoconstriction, and reduce total peripheral resistance. THAM produced vasodilatation and a slight increase in ventricular contraction in animals with a normal acid-base balance. Buckley and Sieker (19), however, reported that THAM administration had little effect in improving the cardiac function of patients in congestive heart failure.

Clark (22) administered 0.3 M THAM (15 ml/g) to dogs with experimentally induced fibrillation. This treatment delayed the development of acidosis and facilitated defibrillation. He advocated the use of a similar dose in clinical cardiac arrest because, in addition to facilitating defibrillation, THAM counteracts acidemia and potentiates pressor drug activity.

Wang *et al.* (166) infused 0.3 M THAM (1.5 mmol) to anesthetized open-chest dogs. There was an immediate and marked increase in coronary sinus outflow and a slight increase in myocardial contractility with no effect on cardiac output or blood pressure. These changes were associated with a rise in the pH and a fall in pCO_2 of the coronary sinus blood and an increase in coronary oxygen A-V difference. The coronary response to THAM administration, which is not produced by equivalent injections of $NaHCO_3$, may be associated with a fall in intracellular $[H^+]$ concentration. Tanaka *et al.* (159) used THAM to counteract the acidosis that occurred during total inflow occlusion in dogs at normal temperature and at 25°C. The animals treated with THAM could tolerate in both instances a significantly longer period of inflow occlusion with complete restoration of myocardial activity. Benichoux (9b) made similar observations in animals cooled to 12 to 20°C. Gollan *et al.* (41) demonstrated that THAM administration improved the response of the hypothermic heart to perfusion with venous blood.

Hinshaw *et al.* (48) observed vasodilatation in the peripheral vessels of the dog after THAM administration. The infusion of THAM, preceded by an injection of endotoxin, resulted in a marked decrease in vascular resistance of the isolated

leg and kidney as well as in the totally perfused animal. There was a significant increase in venous return and a marked fall in hematocrit, due primarily to the reabsorption of tissue fluid. However, the interpretation of these observations is difficult since large amounts of *E. coli* endotoxin had been administered to all preparations prior to the THAM infusion, and control studies with $NaHCO_3$ were not performed.

Ligou *et al.* (68) demonstrated that THAM prevents or corrects the severe pulmonary hypertension which develops during hypercapnia. No measurable changes were observed in pulmonary artery or vein pressures, or in calculated pulmonary resistance following THAM administration during apneic oxygenation. The constancy of pulmonary pressures is particularly remarkable in view of the fluid load administered. Ligou pointed out that THAM administration had prevented both the stimulation of the sympatho-adrenal system and the rise in $[H^+]$ of body fluids which ordinarily accompany the pulmonary hypertension of hypercapnic acidosis. It might also be postulated that THAM produces a vasodilatation of the segments of the pulmonary vascular bed which are constricted when hypercapnic acidosis is present. However, even if this were the case, the studies of Bergofsky *et al.* (11) have indicated that THAM does not alter in dog or in man the pulmonary artery pressor effect due to hypoxia.

I. Cerebral circulation

Inhalation of gas mixtures rich in CO_2 produces a vasodilatation of cerebral vessels and an increase in cerebral blood flow which is manifested by a rise in cerebrospinal fluid pressure. The observations of Nahas (88) demonstrated that the administration of THAM during apneic oxygenation prevented the increase in cerebrospinal fluid pressure which accompanies CO_2 retention. Dos *et al.* (28, 30, 31) extended these observations in similar experiments during which the following solutions were administered: THAM titrated to pH 7.40, 0.6 M *d*-mannitol, and 30% urea in 10% invert sugar (5.3 M). These preparations, which exerted an osmotic effect, did not correct the intracranial hypertension of hypercapnic acidosis. Its correction required the restoration of normal pH not only in blood but in other body fluids as well. The maintenance of a normal blood pH in the presence of an elevated $PaCO_2$ with an infusion of Na_2CO_3, for example, did not prevent a rise in cerebrospinal fluid pressure during apneic oxygenation (54, 94).

The site of THAM's action on the cerebral circulation during CO_2 load is still to be determined. The action seems to be a local one. Clark (22) studied the effect of a rapid infusion of 0.3 M THAM (1 ml/kg) on cerebral oxygen availability in dogs breathing 100% O_2. Oxygen tension, measured with permanently implanted platinum cathodes, dropped by 40% and did not return to its previous level until 15 minutes later. Clark suggested that, since the effect of THAM is of considerably longer duration than the time required to neutralize a single injection, it may be that THAM penetrates the vessel walls and induces a greater fall in the pCO_2 or $[H^+]$ locally than it does in the blood. This hypothesis is consistent with the fact that intracellular sites have a smaller buffer capacity than

extracellular fluids. Meyer *et al.* (84) have also reported that the administration of THAM during CO_2 narcosis resulted in some electroencephalographic improvements associated with an increase in pH and a fall in brain pCO_2. The assumed rapid rate of intracellular penetration of THAM in the brain would contrast with its slow entry into the cerebrospinal fluid (52, 53, 54).

VII. CONCLUSION

THAM is a titrating agent *in vivo* as well as *in vitro*, and its ionized fraction acts in the kidney as a non-reabsorbable cation. Since, at normal blood pH, THAM is 70% ionized, the larger part of the compound remains in the extracellular fluid and is rapidly excreted in the presence of a functional kidney. By reducing the pCO_2 in the extracellular space, THAM could reduce intracellular pH as well. However, such a reduction of intracellular pCO_2 is limited by THAM's depressant effect on ventilation, an effect which rapidly restores to normal and then elevates plasma pCO_2 in the presence of an alkaline pH. The fraction of THAM which penetrates into the cells is, at intracellular pH, 90% ionized and may "overtitrate" the intracellular spaces. As ionized THAM accumulates in the cell, K^+ moves out in order to restore the normal Donnan ratio. In addition to this action on K^+ migration, THAM does not act as a simple buffer inside the cell but most likely interacts with enzyme systems. These interactions remain to be determined and distinguished from the pH effect of THAM. Compounds similar to THAM which would penetrate at a relatively slow rate into cells and modify intracellular pH would be very useful (90) in experimental work in order to alter the activity of specific enzymes. However, their ready availability is unlikely, primarily because of the instability of most organic compounds *in vivo*. Meanwhile, THAM is a very useful tool for the physiologists and pharmacologists who wish to study the effects of acid-base balance alterations. When used judiciously it has also been helpful in clinical medicine. During acidosis, severe metabolic disturbances overwhelm homeostatic mechanisms and many forms of therapy become ineffective. The administration of THAM, by restoring pH of body fluids to normal, potentially can provide optimum conditions for the restoration of homeostasis and for standard therapeutic procedures.

ACKNOWLEDGMENTS

This work was supported in part by grants from the National Institutes of Health, The United States Army Medical Research and Development Command, the National Aeronautics and Space Administration, and Abbott Laboratories. The author gratefully acknowledges the cooperation of Dr. R. K. Richards, Dr. K. J. Frederick, and Dr. S. J. Anderson and Miss Ann Dalziel from the Abbott Research Laboratory as well as the cooperation of Dr. R. J. Hickey and Dr. J. A. Riddick from the Commerical Solvents Corporation Research Laboratory. The assistance of Mariagnes Verosky and Iris Schwartz in the editing and reviewing of the manuscript is also gratefully acknowledged.

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