vaginal secretions from patients with carcinoma of the cervix are in progress in an effort to determine if specific components can be detected which may be utilized in the immunological diagnosis of the disease.

Summary. Vaginal secretion from 55 healthy adult volunteers were analyzed with the double diffusion-precipitin test using antisera to serum proteins and antisera to bronchial secretions, as a preliminary study in search of an immunological means of detecting cancer of the cervix. Approximately one-half of the specimens contained detectable amounts of serum proteins, while 70% reacted with the anti-sputum sera. Of these, 18 specimens (32.7%) reacted with the anti-sputum serum absorbed with normal human serum proteins. Characterization of the components reacting with the antisera was achieved with the immunoelectrophoretic study, and the immunoelectrophoretic patterns of vaginal secretions were compared with the patterns of gastric juices and of bronchial secretions. The 3 secretions had components having cross-reactivities although their physico-chemical properties were different. Use of antisera to vaginal secretions and application thereof to pathologic vaginal secretions are indicated.

1. Lawson, J. G., *J. Obst. Gynec. Brit. Emp.*, 1959, v66, 946.

2. Rauramo, L., *Scand. J. Clin. Lab. Invest.*, 1959, v11, 185.

3. Lawson, J. G., *J. Obst. Gynec. Brit. Emp.*, 1960, v67, 305.

4. Heiskell, C. L., Wada, T., Stempien, S. J., Fukuda, M., Nakagawa, S., Yachi, A., Dagradi, A., Carpenter, C. M., *Gastroenterol.*, 1961, v40, 775.

5. Ibayashi, J., Anzai, T., Hood, J. E., Hyde, L., Carpenter, C. M., to be published.

6. Anzai, T., Ibayashi, J., Hyde, L., Carpenter, C. M., to be published.

7. Scheidegger, J. J., *Intern. Arch. Allergy*, 1955, v7, 103.

8. Hollander, F., Horowitz, M. I., *Gastroenterol.*, 1962, v43, 75.

Received January 23, 1963. P.S.E.B.M., 1963, v113.

Determination of Tris-(Hydroxymethyl) Amino Methane (THAM).* (28275)

J. STRAUSS, K. BERNATH AND S. A. KAPLAN (Introduced by Robert Ward)
Childrens Hospital of Los Angeles and Department of Pediatrics, University of Southern California, Los Angeles

Since the original description of the effects of administration of the organic buffer Tris-(hydroxymethyl) amino methane (THAM) *in vivo*(1) considerable interest has arisen in its potential usefulness as a pharmacological agent(2). The fact that administration of large doses of Tris-(THAM) may cause serious reactions makes desirable the study of its absorption and excretion. Determination of plasma and urine levels of this compound has been limited, however, by lack of a simple, reliable method.

To the authors' knowledge, 5 methods have been described for the determination of Tris-(THAM). 1. Accounting of C-14 labeled Tris-(THAM)(3). 2. Oxidation by potassium dichromate(4). 3. Oxidation to ammonia by alkaline periodate(5). 4. Conversion to ammonia and determination with a modified sodium phenate method(6). 5. Determination by the naphthoquinone method for amino acids(6).

Some of these methods are of limited value for general use, since they require elaborate equipment. Of the simpler methods, details of the procedures are lacking, as are data on the validity of their application to plasma or urine.

This paper outlines a method based on that of Rosen(4) and presents results of the application of this modified method and its evaluation with respect to potentially interfering substances.

* This work was supported by a grant from Nat. Inst. Health.

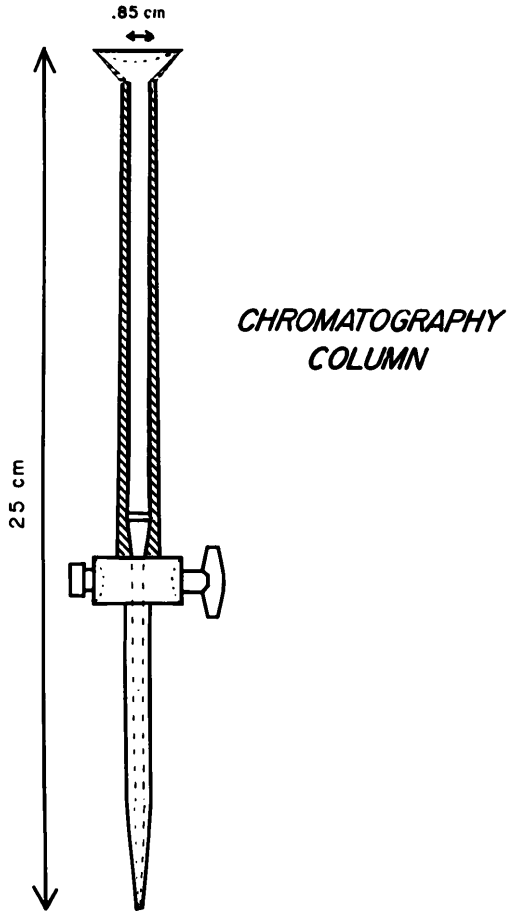

FIG. 1. Glass chromatography column specially designed for determination of Tris-(THAM).

Method. This method is based on the non-specific oxidation of amines by potassium dichromate after removal of acidic reactants by column chromatography. The disappearance of the yellow color of the dichromate as the amines are oxidized is proportional to the concentration of amines.

I. Description of method: A. Reagents. 1. Resin: Dowex 50-X8 (60-100 mesh, H^+ form). 2. 6 N sulfuric acid. 3. 0.2% potassium dichromate in 20 N sulfuric acid. 4. 1.0% and 0.01% Tris-(hydroxymethyl) amino methane (THAM) standard solutions in water. 5. 10% zinc sulphate in water. 6. 0.5 N sodium hydroxide solution in carbon dioxide free water.

B. Glassware. *For plasma,* the small chromatography column (i.d. 0.85 cm, 24.7 cm length) preferably with a coarse fritted glass filter, was found satisfactory. A short length of rubber tubing was applied to the lower end of the column and rate of flow regulated by a pinch clamp. Although this arrangement was satisfactory, a glass chromatography column, specially designed for accuracy and ease in handling, was used (Fig. 1). *For urine,* a larger chromatography column (i.d. 1.9 cm, 33.7 cm length) or a glass stoppered 50 ml volumetric cylinder was used.

C. Procedure. 1. Method for Plasma. a. Protein precipitation: To 0.4 ml plasma add 0.4 ml zinc sulphate and 0.4 ml sodium hydroxide. Mix by tapping and centrifuge at 2500 rpm for 10 minutes. b. Chromatographic separation: Introduce 0.2 g moist resin into the small chromatography column, close the stopcock or pinch clamp and add 0.5 ml supernatant plus 0.5 ml water. Let stand for 3 minutes, then drain through the resin. Wash the resin 3 times with water added up to the top of the column. After the last water washing, close the stopcock and add 1.0 ml 6 N sulfuric acid. Let stand for 2 minutes, then collect the effluent in a 1.0 × 10 cm tube. c. Amine oxidation: Add 0.3 ml potassium dichromate, cover with marbles and put tubes in a boiling water bath for 30 minutes. Remove, cool for 5 minutes in room air, and transfer to a photometer tube. Carry blank (1 ml 6 N sulfuric acid) and standard (0.1 mg Tris-(THAM) in 1.0 ml water) through oxidation. Read at 400 mμ in photometer. d. Calculation:

$$\frac{\text{O. D. Blank} - \text{O. D. Unknown}}{\text{O. D. Blank} - \text{O. D. Standard}} \times 60 = \text{mg/100 ml plasma.}$$

Optimum range: 20-90 mg/100 ml.

2. Method for Urine. a. Chromatographic separation: To 1.0 ml of urine add 2.0 ml water. Mix. Introduce this diluted urine into the large chromatographic column or volumetric cylinder containing 1.0 g resin. *For cylinder*: Shake constantly for 3 minutes. Fill with water. Aspirate the supernatant and leave a few millimeters of fluid above the resin. Add 10 ml water, stopper and shake for 3 minutes. Fill with water to the 50 ml

mark and aspirate to a few millimeters of the resin. Add 5 ml 6 N sulfuric acid and shake for 2 minutes. Fill with water to the 25 ml mark, stopper and shake for 2 minutes. Pipette 5 ml supernatant into a 1.5 × 12 cm tube. *For chromatographic column*: Same procedure without shaking. b. Amine oxidation: Add 3 ml potassium dichromate solution. Cover with marbles and put in boiling water bath for 30 minutes. After removal from the water, cool for 5 minutes and transfer to a photometer tube. Carry blank (5 ml 6 N sulfuric acid) and standard (5 mg Tris-(THAM) in 3.0 ml water) through oxidation. Read at 400 mμ in photometer. c. Calculation:

$$\frac{\text{O. D. Blank} - \text{O. D. Unknown}}{\text{O. D. Blank} - \text{O. D. Standard}} \times 5 = \text{mg/ml urine.}$$

Optimum range: 2-7.5 mg/ml.

II. Discussion of method. A. Volume of original sample: With the dose of Tris-(THAM) recommended for clinical use, a plasma sample of 0.4 ml contains measurable amounts of this substance. The described volumes of diluted plasma supernatant and urine permit adequate mixing with the resin. B. Protein precipitation. The method as originally described(4) recommends protein precipitation with Trichloroacetic acid (TCA) and subsequent ether extraction of TCA to avoid interference with color development. Since zinc sulfate and sodium hydroxide do not interfere with color development, as protein precipitants, they are preferable to TCA. In addition, the study of renal excretion of Tris-(THAM) is simplified, since Inulin and para-aminohippurate may be measured in the same supernatant. C. Photometer: Maximum sensitivity for dichromate is found in the ultra-violet range; however, there is adequate sensitivity at 400 and 450 mμ. Therefore, photometers or colorimeters available in most laboratories can be used. In this study, the Coleman Jr. Spectrophotometer was used. D. Interfering substances: Tris-(THAM)-free urine, and plasma, analyzed by this method, produced no change in optical density. 1. Amino acids and amines: 2.5 μmol of the following substances were buffered to pH 7.5 with phosphate buffer and made up to 20 ml with water: Alanine, beta-alanine, arginine, asparagine, aspartic acid, alpha-amino-n-butyric acid, beta-amino-iso-butyric acid, carnosine, citrulline, cystathionine, cystine, glucosamine, glutamic acid, gamma-amino-butyric acid, glutamine, ethanolamine, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, phosphoethanolamine, proline, sarcosine, serine, taurine, threonine, tryptophan, tyrosine, ammonia sulfate, valine and choline chloride. Aliquots of this solution comparable to amounts normally present in plasma and urine, were taken through the analytical procedure. There was no interference with the determination. It was concluded that except in unusual circumstances, the presence of amines or amino acids in plasma and urine does not interfere with this method of analysis. 2. PAH, in amounts similar to those obtained in urine and plasma during clearance studies, was added to Tris-(THAM)-containing solution, and analyzed by the present method. Tris-(THAM) recoveries after PAH addition were higher than recoveries before PAH addition; the increase equaled 35% of the added PAH. 3. Penicillin, in amounts of 10 and 50 I.U./ml was also analyzed by the present method. Addition of Penicillin did not change Tris-(THAM) recoveries. E. Recovery: Average recovery for urine method ranged from 98.4% to 100.2% with standard deviation (S. D.) from ± 2.83% to ± 2.98%; average recovery for plasma method ranged from 99.7% to 101.9% with S. D. from ± 1.49% to ± 3.36%. The results followed Beer's Law from 0.02 to 0.2 mg with the plasma method and from 0.2 to 7.5 mg with the urine method.

TABLE I. Recoveries of Tris-(THAM) from Water, Plasma and Urine.

Method	No. of samples	mg added	Solution	% recovery	% S.D.
Plasma	10	.100	Water	99.7	± 1.49
"	10	.104	Plasma	101.9	± 3.36
Urine	10	5.0	Water	100.2	± 2.86
"	9	2.5	"	98.4	± 2.83
"	10	5.0	Urine	100.0	± 2.98

Discussion. Specific features characterize each of the 5 known methods for analysis of Tris-(THAM) and make each one more fit for some purposes than for others. The method(3) based on the use of C-14 tagged Tris-(THAM) is the most accurate, but is very costly and is not applicable to humans. Linn and Roberts' titrimetric method(5) is desirable when only minute quantities of sample can be obtained, but it requires standing over night. Clark's methods(6) seem reproducible but they require special equipment.

Though Rosen's method(4) generally is described as unsuitable for blood analysis(2, 6), its modification as described here is applicable to either plasma or urine. It yields average recoveries of over 95% of Tris-(THAM) added to biological fluids. Reproducible calibration curves are obtained, and there is no interference by amines, amino acids or Penicillin at usual levels. It seems that the protein precipitation in the modified method introduces fewer errors than the one described by Rosen(4). In addition, the modified concentration of some reagents and the use of special glassware, might be contributory factors in making this modified method suitable for both blood and urine analysis. The relative simplicity of this method, both in procedure and equipment, makes it desirable for general use.

Summary. 1. A modification of Rosen's method for Tris-(THAM) has been presented. Both Rosen's method and this modification are based on the oxidation of Tris-(THAM) by potassium dichromate after chromatographic separation to remove interfering substances. 2. Tris-(THAM) recoveries of over 95% were obtained without interference from amino acids or Penicillin. There was PAH interference by up to 35% of added PAH. 3. This method is applicable for use with small quantities of plasma and urine following injection of Tris-(THAM) in recommended pharmacological doses. 4. Pending development of a specific chemical test, this method is recommended for general use because of its simplicity and reproducibility.

1. Nahas, G. G., *Science,* 1959, v129, 782.
2. Nahas, G. G., *Pharmacol. Rev.,* 1962, v14, 447.
3. Holmdahl, M. H., Nahas, G. G., *Fed. Proc.,* 1961, v20, 75.
4. Rosen, H., *Ann. N. Y. Acad. Sci.,* 1961, v92, 414.
5. Linn, S., Roberts, M., *ibid.,* 1961, v92, 419.
6. Clark, L. C., Jr., *ibid.,* 1961, v92, 687.

Received January 24, 1963. P.S.E.B.M., 1963, v113.

Blood Changes in Turkeys Associated with a Copper Deficiency.* (28276)

CHARLES F. SIMPSON, R. H. HARMS AND R. L. SHIRLEY
Department of Veterinary Science, Poultry Science and Animal Science, University of Florida, Gainesville

It is known(1) that copper is an indispensable trace mineral constituent of the diet for many species of animals. Gallagher(2), however, was unable to produce an uncomplicated copper deficiency anemia in chicks. The turkey's requirement for copper has not been determined(3).

Until recently is was assumed that erythropoiesis was the only metabolic process in which copper was concerned. It is believed that copper does have a role in connective tissue integrity because copper deficient pigs(4) and chicks(5) died from internal hemorrhage as a consequence of elastic fiber degeneration and aortic rupture.

This study of copper deficiency in turkeys was initiated because many identical histopathological lesions have been described in chicks fed a copper deficient diet(5) and turkeys fed *Beta-aminopropionitrile* (BAPN)

* Supported in part by grants from Nat. Heart Inst., U.S.P.H.S.
† Florida Agri. Exp. Station Journal Series No. 1635.