The methods for analyses of Na and K were those previously described.[2] NH_3 was determined by Sobel, Yuska and Cohen's modification of Van Slyke and Cullen's method.

The cortical extracts used were highly purified, contained very little solid, were free from protein and contained practically no nitrogen. Potent extracts gave a precipitin reaction with serum from refractory dogs, but did not react with normal dog serum. Preliminary experiments, using cortin as the antigen, would indicate that the serum of repeatedly injected animals may have some power of fixing complement.

We wish to express our thanks to Dr. F. A. Hartman for his helpful criticism and advice throughout the course of these experiments.

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Determination of Amino-Nitrogen in Urine.

F. A. TAYLOR. (Introduced by S. R. Haythorn.)

From the William H. Singer Memorial Research Laboratory, Allegheny General Hospital, Pittsburgh, Pa.

Despite the importance of an understanding of amino-acid metabolism, there has been no trustworthy method of amino-nitrogen determination that could be easily applied as a routine in a clinical laboratory. The chief difficulty has been in the preparation of filtrates of blood and urine for the actual titrations. In this note a method is described for the removal of NH_3 and CO_2 from urine filtrates enabling one to determine amino-nitrogen expeditiously by the Sörensen titration. Many determinations may be carried out in parallel with little supervision by the analyst.

The Sörensen[1] titration as described by Northrop[2] is used. The preparation of urine filtrates follows the procedure of Van Slyke and Kirk.[3] Instead of distilling the NH_3 and CO_2 successively *in vacuo,* the filtrates are exposed in shallow layers *in vacuo* in a desiccator over dilute H_2SO_4. This means of collecting or removing NH_3 has been known for a long time and occasional reference is

[2] Thorn, G. W., Garbutt, Helen R., Hitchcock, F. A., and Hartman, F. A., *Endocrin.,* 1937, **21**, 213.

[1] Sörensen, S. P. L., *Biochem. Z.,* 1908, **7**, 45.

[2] Northrop, J. H., *J. Gen. Physiol.,* 1926, **9**, 767.

[3] Van Slyke, D. D., and Kirk, E., *J. Biol. Chem.,* 1933, **102**, 651.

made to it in the current literature. It requires no further effort on the part of the analyst, in contrast to the individual distillations *in vacuo* as described by Van Slyke and Kirk or as further improved by Kirk.[4] Quantitative transfer of solutions from the large inaccessible surface of the flask is likewise avoided.

In preliminary trials it was found that NH_3 could be removed from ammonium chloride in about 3 hours. With urine filtrates, however, 5-7 hours was required for constant titration figures. The alkaline filtrate remains unchanged in the refrigerator for several days so that analyses may be repeated.

Method. Urine filtrates after Van Slyke and Kirk (5 ml.) are placed in 50 ml. beakers with as little exposure to air as possible. The lips of the beakers may be paraffined to facilitate pouring. At the same time 5 ml. of the filtrates are placed in test tubes. Neutral red solution and 1 ml. of formalin are added to each tube. If the solutions remain alkaline, sufficient alkali has been added to set free the NH_3 present. Otherwise more barium hydroxide must be added to the samples in the beakers. The beakers are placed in a vacuum desiccator over broken alundum saturated with dilute (1-3) H_2SO_4.* The pressure is reduced to 20 mm. or less and the desiccator allowed to stand over night. Twelve beakers may be placed in an 8-inch desiccator.

The following morning the beakers are removed, a drop of neutral red indicator solution (0.025%) added to each and the solutions acidified with 0.1 N HCl. Two drops in excess are added to maintain a slight acidity after decomposition of a small amount of barium carbonate and release of CO_2.

The solutions are again evacuated for 1-2 hours, after which they are transferred to test tubes for titration. By using a very fine paraffined tip on the supply of CO_2-free water (discharging 1 ml. in 2.5 sec.) the solutions are removed quantitatively with less than 1.5 ml. of water—the amount lost by evaporation in the desiccator. They are thus brought to the original volume. Neutral and alkaline standards are prepared and the amino-acid solutions are titrated with 0.01 N NaOH. Formalin blank titrations are subtracted.

Especially with highly colored urines, it is difficult to prepare

[4] Kirk, E., *Acta med. Scand.*, 1936, Suppl. **77**, 46.

* The broken alundum (furnished by the Norton Co. and the Fisher Scientific Co.) is prepared by soaking in the H_2SO_4 and is renewed by pipetting the acid over its surface in the desiccator and then removing the liquid that drains to the bottom. If much free liquid is present, there is spattering on evacuation.

several neutral end point standards independently and have them match each other. As a substitute the neutral red indicator solution is diluted 5 times with water and 1 drop added to the alkaline filtrate to serve as standard. One drop of 0.01% phenol red is added and then 1.0 N HCl to neutrality, followed by 5 drops in excess The pH is then 1-2 and the color matches that of the stronger neutral red solution at pH 7. The urine filtrate may be used for this standard since the solution is acid in reaction. It replaces the Northrop neutral standard containing phosphate.

In a series of 63 determinations in triplicate, the variation has averaged 0.02 ml. with a maximum of 0.07 ml. In comparisons with titrations after distillation *in vacuo* the variation has been 0.04 ml. which is the error to be expected from duplicates by that method. Much of the variation is probably caused by inequality in size of drops of indicator solutions so that titrations carried out with independently made standards will vary more than those made with the same standards.

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Hydrogen-Ion Concentration of the Gall Bladder Bile of the Dog.

FRANCES F. BECK,* JOHN C. KRANTZ, JR., MAURICE FELDMAN AND C. JELLEFF CARR.

From the Department of Pharmacology and Research Division of the Department of Gastroenterology, School of Medicine, University of Maryland.

An experimental study was reported[1] on the hydrogen-ion concentration of the bile of the guinea pig. In this series of experiments, effort has been made to determine which constituents of the dog's gall bladder bile are responsible for the fluctuating pH in normal dogs and also upon medication with Extract of Ox Bile, U.S.P.

The hydrogen-ion concentration of the gall bladder bile of normal dogs was determined by means of the glass electrode at 25° and an analysis of the principal constituents was carried out according to Douglas-Sauermann's[2] method. The results on 8 dogs are shown in Table I.

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* Emerson Fellow in Pharmacology.

[1] Krantz, J. C., Jr., Feldman, M., Morrison, S., and Carr, C. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 48.

[2] Douglas-Sauermann, A. G., *Z. Physiol. Chem.*, 1935, **231**, 92.