bent in any desired position, its general utility must be apparent. Both the shaft and the lamp may be sterilized in solutions, or the lamp may be so treated and the shaft boiled. The metal case which covers the storage battery or dry cell is covered with sterile gauze, or a sterile muslin sheath, made to fit, is drawn over the case before it is placed with the other instruments. This affords perfect and safe control of the instrument.

The device so arranged gives excellent illumination in all cases in which deep ocular inspection is desired during the course of the operation. This lamp is also of value in the transillumination of the stomach, gall-bladder, biliary ducts, duodenum and other hollow viscera. The instrument has been made for me by the Paul V. Muckle Instrument Company of Denver.

612 Empire Building.

A PERMANENT PREPARATION OF UREASE, AND ITS USE FOR RAPID AND ACCURATE DETERMINATION OF UREA*

DONALD D. VAN SLYKE AND GLENN E. CULLEN, NEW YORK

The procedure described below permits any one with rudimentary laboratory experience to complete within half an hour an accurate determination of the urea content of the urine, blood or spinal fluid. The apparatus required consists merely of test-tubes, burets and a suction-pump such as can be attached to any tap with fair water-pressure.

The methods for urea determination have until recently been of two classes, dependent respectively on either (1) the hypobromite reaction, or (2) the principle of transforming the urea into ammonia by heating to 150 C. (302 F.) in acid solution. The hypobromite methods are notoriously inaccurate. The heating methods, particularly that of S. R. Benedict, are, for urine at least, accurate, but not suited for use outside of a laboratory.

Marshall has recently introduced a third principle which offers an opportunity for obtaining results equal in accuracy to the best laboratory methods, with a technic almost as simple as that of the hypobromite procedure. Marshall utilized an enzyme, urease, discovered in 1909 by Takeuchi in the water extract of the soja or soy bean, which converts urea into ammonium carbonate. It acts at room temperature, forms ammonia from nothing except urea, and is not interfered with by glucose or anything else likely to be encountered in physiologic fluids. Marshall's extract has, however, two disadvantages compared with the chemical reagents available: it requires a number of hours to react quantitatively, and it loses its activity on standing.

These disadvantages have been overcome by the preparation of the enzyme in the form of a soluble and very active dry powder. Soja bean meal is extracted with 5 parts of water and the extract poured into 10 volumes of acetone. The precipitate containing the enzyme is dried, and obtained as a powder which maintains its activity indefinitely. The enzyme prepared in this form can be obtained from the Arlington Chemical Company, of Yonkers, N. Y.

Properties of the Urease.—The enzyme preparation dissolves readily in water, forming an opalescent solution. Under given conditions, a given amount of it will decompose a definite amount of urea per minute, and no more, regardless of how much excess urea may be present. The preparation is most active at a temperature of approximately 55 C. (131 F.) and in a perfectly neutral solution. Between 10 and 50 C. (50 and 122 F.), increasing the temperature over any 10 degree interval doubles the rate at which the preparation acts. Heavy metals, or acid in a relatively slight concentration, destroy the activity. The urease is not quite so sensitive toward an alkaline reaction, but its activity is decreased by even weak bases. Ammonium carbonate depresses the activity to about one-eighth the optimum, so that the action of the enzyme is self-retarding. The retarding effect of the ammonium carbonate formed can be neutralized and conditions for optimum activity obtained by the addition of potassium dihydrogen phosphate (potassium acid phosphate, KH_2PO_4), and dipotassium hydrogen phosphate (neutral potassium phosphate, K_2HPO_4), in equimolecular proportions. This phosphate mixture also acts as a stabilizer to solutions of enzyme. While the latter ordinarily begin to lose strength rapidly after from one to five days, a solu-

tion containing 10 per cent. of the enzyme preparation in 0.2 molecular $\text{KH}_2\text{PO}_4 = \text{K}_2\text{HPO}_4$ lost only 3 per cent. of its activity in three weeks when kept under toluene at from 25 to 30 C. (77 to 86 F.) in a place exposed daily to direct sunlight.

Preparation of Enzyme Solution.—Two gm. of the enzyme preparation, 0.6 gm. of dipotassium hydrogen phosphate, and 0.4 gm. of potassium dihydrogen phosphate are stirred up with a rod in 10 c.c. of water. The enzyme preparation dissolves in about a minute, forming an opalescent solution. A few floccules of insoluble matter may remain, but the active enzyme all goes into solution at once. The urease can be obtained from the manufacturers in 1-gm. portions already mixed with the proper amounts of phosphate, so that it is merely necessary to dissolve the mixture in 10 c.c. of water. The acid phosphate serves a double purpose: it accelerates the enzyme action, and renders the enzyme solution more stable. If the latter is covered with toluene it will ordinarily hold its activity for a fortnight, but it is safer to use fresh solutions.

Analysis of Human Urine.—The procedure consists in treating the urine sample with urease, aerating the ammonia

Apparatus for determining the urea content by means of urease.

formed into fiftieth-normal acid, and titrating back the excess acid.

The details are carried out as follows:

1. Dilute 5 c.c. urine to 50 c.c.
2. Measure into Tube A 5 c.c. diluted urine, 1 c.c. enzyme solution, 1 drop caprylic alcohol.
3. Close A with stopper shown in figure, and let stand fifteen minutes for enzyme to act.
4. Measure into tube B 25 c.c. fiftieth normal hydrochloric acid or sulphuric acid, 1 drop 1 per cent. sodium alizarinate indicator, 1 drop caprylic alcohol.
- Connect A and B as shown in figure.
5. At the end of fifteen minutes pass the air-current as indicated for one-half minute.
6. Then open A and pour in 4 to 5 gm. of dry potassium carbonate measured from a spoon.
7. Close A at once and aerate again till all ammonia is driven over into the acid in B.
8. Titrate excess acid in B with fiftieth-normal sodium hydroxid.

The caprylic alcohol (Kahlbaum's "Octylalkohol, Sekundär I") serves to prevent foaming during the aeration. The purpose of the preliminary half-minute aeration (paragraph 5 above) is to drive over into the acid in B any ammonia that may have escaped from the urine into the air space in A. The time required for the final aeration depends on the rate at which the air-current is passed through the solutions,

* From the Laboratories of the Rockefeller Institute for Medical Research.

and must be determined by trial for the particular pump or pressure system that is utilized. With a central vacuum, pressure, or an efficient pump which will give as rapid a current as the apparatus will stand, it is possible to drive off all the ammonia in five minutes, while with a slow pump half an hour may be necessary. The number of cubic centimeters of fiftieth-normal acid neutralized by the ammonia (i.e. 25 minus the c.c. of alkali used in Process 8) is multiplied by the factor 0.056 to give the number of grams of urea plus ammonia nitrogen per hundred cubic centimeters of urine. The ammonia alone may be determined at the same time with the ammonia plus urea, using the same technic, except that 5 c.c. of *undiluted* urine, no urease, and the factor 0.0056 are used for determination of the ammonia nitrogen alone. The ammonia tubes are connected in series with those for the urea determination, using the same air current for all. One can run as many as eight pairs of tubes in series on one current, taking care only to disconnect the series in the middle, to prevent back suction, before the pump is disconnected.

Determination of Urea in Blood, Spinal Fluid, etc.—The urease is particularly valuable in permitting a simple and accurate determination of urea in the blood, because its action is so specific that it attacks none of the other constituents, and the blood has to undergo no preliminary treatment for removal of the latter. Three c.c. of fresh blood or fluid, measured with an accurate pipet, are run into a 100-c.c. test-tube containing 1 c.c. of 3 per cent. potassium citrate, to prevent clotting; 0.5 c.c. of the urease solution and 2 or 3 drops of caprylic alcohol are added. After ten minutes 5 c.c. of saturated potassium carbonate solution are added, the ammonia is driven by aeration into 15 c.c. of hundredth-normal acid, and the excess acid is titrated back with hundredth-normal sodium hydroxid. Each cubic centimeter of hundredth-normal acid neutralized indicates 0.01 per cent. (grams per hundred cubic centimeters) of urea in the blood, or 0.00467 per cent. of urea nitrogen. In case the blood should be one of the rare samples containing over 0.15 per cent. of urea, all the acid will be neutralized, and it will be necessary to repeat the determination, using a sample of only 1 c.c. Fresh blood contains so little ammonia that it can be disregarded.

Technic of Aeration.—The technic is essentially that of Folin, with some minor alterations to adapt it to the somewhat changed conditions. The tubes do not have to stand heat, and consequently can be made of very heavy and durable glass. A convenient holder (shown in the illustration) for them is easily made from a block of wood 9 by 5 by 4 inches, by drilling in it eight holes, each $1\frac{1}{8}$ inches in diameter and 3 inches deep.

To prevent foaming, caprylic alcohol is by far the most efficient agent with which we are acquainted. With it one has no trouble at all, even in very rapid aeration of blood. In its absence one can use amyl alcohol or kerosene, although these do not guarantee the same immunity from annoyance and lost analyses. In view of the small amounts of caprylic alcohol required (from 2 to 5 drops for an analysis) its use is not expensive.

The inlet tube for air should always reach to the bottom of the solution.

The most convenient rubber tubing for the connections is the thick-walled, soft stethoscope tubing.

The apparatus, arranged as shown in the accompanying illustration, is so simple that it can be made in a laboratory, but can be obtained, with the block holder shown, suction pump, and a set of especially heavy test-tubes, from Emil Greiner & Co., 45 Cliff Street, New York.

In connection with the subject the following references will prove of interest:

- Takeuchi: *Jour. Coll. Agric.*, Tokyo, 1909, Part 1.
Armstrong: *Proc. Roy. Soc.*, 1912, lxxxv, Series B, p. 109; lxxxvi, 328, 561, 1913.
Marshall: *Jour. Biol. Chem.*, 1913, xiv, 283; 1913, xv, 487.
Folin: *Jour. Biol. Chem.*, 1912, xi, 493.
Van Slyke and Cullen: Preliminary reports, *Proc. Soc. Exper. Biol. and Med.*, Dec. 17, 1913; *Proc. Soc. Biol. Chem.*, Dec. 29, 1913.

Distribution of Sexes in Manila.—In Manila males greatly exceed females in number. At present there are 149,397 males as against 131,608 four years ago, and 117,546 females as against 102,801 four years ago, that is, about 32,000 more males than females. There are only 3,626 widowers in Manila, while the number of widows is 10,961. Prosperous conditions offering good employment account for the movement to the metropolis of the islands.

New and Nonofficial Remedies

THE FOLLOWING ADDITIONAL ARTICLES HAVE BEEN ACCEPTED BY THE COUNCIL ON PHARMACY AND CHEMISTRY OF THE AMERICAN MEDICAL ASSOCIATION. THEIR ACCEPTANCE HAS BEEN BASED LARGELY ON EVIDENCE SUPPLIED BY THE MANUFACTURER OR HIS AGENT AND IN PART ON INVESTIGATION MADE BY OR UNDER THE DIRECTION OF THE COUNCIL. CRITICISMS AND CORRECTIONS ARE ASKED FOR TO AID IN THE REVISION OF THE MATTER BEFORE PUBLICATION IN THE BOOK "NEW AND NONOFFICIAL REMEDIES."

THE COUNCIL DESIRES PHYSICIANS TO UNDERSTAND THAT THE ACCEPTANCE OF AN ARTICLE DOES NOT NECESSARILY MEAN A RECOMMENDATION, BUT THAT, SO FAR AS KNOWN, IT COMPLIES WITH THE RULES ADOPTED BY THE COUNCIL.

W. A. PUCKNER, SECRETARY.

EREPTON.—Erepton is a product prepared by the digestion of meat and consisting largely of the amino-acids thus produced. One hundred Gm. of erepton contains about 12 Gm. of nitrogen (the exact nitrogen content is stated on each package) in a form which does not give the biuret reaction.

Actions and Uses.—Erepton is said to be capable of supplying the nitrogen requirements of the animal organism. It is claimed to be non-toxic and non-irritating and to be satisfactorily absorbed when introduced into the rectum. Erepton is said to be useful in cases in which it is necessary to substitute a perfectly digested food for the product of natural digestion in cases of gastric or intestinal indigestion and for the purposes of rectal alimentation.

Dosage.—Erepton is usually administered as a nutrient enema in a solution having the temperature of the body. It may be used from three to four times daily. The following prescription is best adapted for the purpose:

Erepton	20.0 Gm. ($\frac{3}{4}$ ounce)
Pure maltose	20.0 Gm. ($\frac{3}{4}$ ounce)
Water	200.0 Gm. (7 ounces)

Should it appear desirable, 10 Gm. (150 grains) of fat may be added to the preceding in the form of olive-oil or butter. Erepton may be given in porridges, vegetables or soups.

Manufactured by Farbwerke, vorm. Meister, Lucius and Bruening, Höchst a. M., Germany (Farbwerke-Hoechst Co., New York). No U. S. patent. U. S. trademark No. 88,403.

Erepton is prepared by the successive action of pepsin-hydrochloric acid, trypsin and erepsin on meat freed from fat and tendon; the end-product is then desiccated.

Erepton is a brownish, hygroscopic powder, easily soluble in water and having an odor and taste suggestive of meat extract.

SEROBACTERINS.—(See THE JOURNAL, Feb. 7, 1914, p. 457.) The Council has at present no means of determining the identity and purity of serobacterins; they must be used on the guarantee of the manufacturer alone and they must be used, therefore, with due caution.

H. K. Mulford Co., Philadelphia.

Acne Serobacterin, Mulford (Sensitized Acne Vaccine, Polyvalent).—Marketed in packages of four syringes, graduated as follows: Syringe A, 100 million killed sensitized acne bacilli; Syringe B, 200 million killed sensitized acne bacilli; Syringe C, 400 million killed sensitized acne bacilli; Syringe D, 800 million killed sensitized acne bacilli.

Coli Serobacterin, Mulford (Sensitized Coli Vaccine).—Each package contains four syringes of Coli Serobacterin graduated as follows: Syringe A, 250 million killed sensitized *Bacillus coli*; Syringe B, 500 million killed sensitized *Bacillus coli*; Syringe C, 1,000 million killed sensitized *Bacillus coli*; Syringe D, 2,000 million killed sensitized *Bacillus coli*.

Neisser Serobacterin, Mulford (Sensitized Gonococcic Vaccine).—Each package contains four syringes of Neisser serobacterin graduated as follows: Syringe A, 250 million killed sensitized gonococci; Syringe B, 500 million killed sensitized gonococci; Syringe C, 1,000 million killed sensitized gonococci; Syringe D, 2,000 million killed sensitized gonococci.

Pneumo Serobacterin, Mulford (Sensitized Pneumococcic Vaccine).—Each package contains four syringes of Pneumo Serobacterin graduated as follows: Syringe A, 250 million killed sensitized pneumococci; Syringe B, 500 million killed sensitized pneumococci; Syringe C, 1,000 million killed sensitized pneumococci; Syringe D, 2,000 million killed sensitized pneumococci.

Staphylo Acne Serobacterin, Mulford (Sensitized Staphylo Acne Vaccine).—Each package contains four syringes of Staphylo Acne Serobacterin graduated as follows: Syringe A, 500 million killed sensitized staphylococci, 100 million killed sensitized acne bacilli; Syringe B, 1,000 million killed sensitized staphylococci, 200 million killed sensitized acne bacilli; Syringe C, 2,000 million killed sensitized staphylococci, 400 million killed sensitized acne bacilli; Syringe D, 4,000 million killed sensitized staphylococci, 800 million killed sensitized acne bacilli.