SCIENTIFIC SECTION, AMERICAN PHARMACEUTICAL ASSOCIATION

UREASE.*

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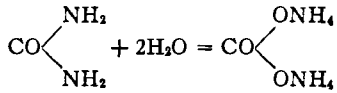
Urease is an enzyme found in numerous bacteria, fungi, and in certain higher plants. It readily converts urea into ammonium carbonate and, on this account, is not only interesting, but extremely useful as a diagnostic agent.



The presence of urease in soy bean (*Glycine hispida*) was first observed by Takeuchi, and was afterwards applied to the quantitative estimation of urea by E. K. Marshall, Jr., Ph.D., of the Physiological Chemical Laboratory of the Johns Hopkins Medical School.

To estimate the amount of urea in urine, it is only necessary to determine, with decinormal hydrochloric acid and methyl orange, the degree of natural alkalinity of a portion of the specimen that has not been treated, and compare this, as to alkalinity, with an equal quantity of the same specimen which has been treated with the enzyme. The difference, of course, represents the ammonium carbonate formed by the breaking up of the urea present; the amount of this urea can be readily ascertained by calculating its equivalent in ammonium carbonate as hereinafter shown.

This method yields accurate results by a procedure much simpler than any heretofore employed; the other accurate methods in general use all require an independent determination of the pre-formed ammonia, and generally are too complicated to be favored for rapid clinical work. It is because of such difficulties and complications that the various older methods, which require the decomposition of urea by an alkaline solution of sodium hypobromite and the measurement of the nitrogen evolved, are still used for clinical purposes; these hypobromite methods, however, as is well known, are very inaccurate, the results being vitiated by the presence of unknown quantities of ammonium salts.

In developing the urease method, Dr. Marshall first obtained the enzyme, in an aqueous extract of ground soy beans, by treating them with water, precipitating much of the protein matter with hydrochloric acid, and clearing the solution by filtration; this solution was preserved with toluol, but could not be satisfactorily kept longer than about a week.

The soy bean extract yields accurate results, but possesses certain disadvantages, especially when rapid estimations are desired. As previously stated, it deteriorates on standing and must, on this account, be freshly prepared at short intervals, and, to ascertain the extent of its inherent effect on methyl orange, a preliminary determination or titration of the extract, itself, must be made. Besides these objections, the larger amount of soy bean extract required to produce rapid conversion of urea makes a solution that becomes very cloudy upon the addition of acid, due to the precipitation of more of the proteins; this cloudiness interferes with a close observation of the color change and thus causes a sacrifice of the

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accuracy that follows sharp end reactions. These disadvantages led to the preparation of urease.

Urease is a fine, almost white powder with little taste or odor; it is soluble in slightly alkaline water and presents the urea-converting enzyme of soy bean in a condition approximating purity. It is practically free of water-soluble proteins that are precipitated by hydrochloric acid and of proteins that are insoluble in water. Aqueous solutions deteriorate after standing a few days.

For the estimation of urea, urease possesses the following advantages over the soy bean extract: (*a*) It is practically neutral to methyl orange, which characteristic renders unnecessary the blank determination that is required for the alkaline soy bean extract; (*b*) it can be preserved in dry form, suitable for immediate use, indefinitely; (*c*) it is readily soluble in urine; (*d*) it gives a clear end reaction, since it yields very little cloudiness on the addition of acid during titration, and, consequently, can be used in larger proportions without the sacrifice of accuracy that follows the use of increased quantities of the soy bean extract.

The procedure for using urease is identical with the original technic of Marshall, excepting that 25 milligrammes (one tablet) of this dry substance are substituted for two cubic centimetres of the liquid soy bean extract. For the more rapid decomposition of the urea, two modifications of his original method are suggested by Dr. Marshall: first, the use of a greater proportion of the enzyme to the urine treated; second, an increase of temperature to 35°–40° C., during the catalytic process.

DIRECTIONS FOR USING UREASE.

Apparatus and material: Besides the urease, which for convenience in the test may be had in 25 milligramme tablets, there are required four 200 cubic centimeter Erlenmeyer flasks with cork stoppers; one 50 cubic centimeter glass-stoppered burette; one 5 cubic centimeter bulb pipette; one small glass mortar; 100 Cc. solution of methyl orange; 1000 Cc. decinormal hydrochloric acid and 50 Cc. toluol.

For Urine.—Put 1 or 2 cubic centimeters of toluol into each of two Erlenmeyer flasks of 200 cubic centimeter capacity. Into one of the flasks, introduce exactly 5 cubic centimeters of a specimen and 100 cubic centimeters of distilled water; stopper the flask with a cork. Place 25 milligrammes urease or crush a tablet of same weight in a small glass mortar, and dissolve it in about 5 cubic centimeters of water. Transfer this solution, without loss, into the other flask containing toluol, and rinse the mortar with several portions of distilled water until about 100 cubic centimeters have, in this way, been added to the contents of the second flask; now add exactly 5 cubic centimeters of the urine specimen and stopper with a cork.

Agitate each flask thoroughly, to mix its contents, and allow both flasks to stand at room temperature over night, or, at least, eight hours, for the slow method. Should it be desired to use the rapid method, the procedure is exactly the same, excepting that two tablets are used for a test and the mixture containing the specimen is digested at about 40° C. for one hour.

The test may be completed in fifteen minutes by using 1 cubic centimeter of urine specimen, 50 milligrammes of urease, and digesting the mixture at 40° C. for about fifteen minutes. The factor would in this case be the whole number, 3, instead of the decimal, .6 (see last paragraph).

After the lapse of the proper time, the two solutions should be titrated to a distinct pink color with decinormal hydrochloric acid, using methyl orange as an indicator.

Should it be desired to make a large number of determinations at one time,

the 5 cubic centimeter control portions of the several specimens may be titrated, as to existing alkalinity, with decinormal hydrochloric acid and methyl orange, one after another, in the same flask, at the time the portions to be examined for urea are prepared in separate flasks, for digestion; also, in such cases, a sufficient amount of urease for all the determinations may be rubbed up in a mortar, with a measured quantity of distilled water, and an aliquot portion, with 100 cubic centimeters of distilled water, added to each of the separate specimens that are to be treated with the enzyme.

For Blood.—Either of two procedures may be used. One is the method given by Dr. Marshall in his paper, "A New Method for the Determination of Urea in Blood," published in the *Journal of Biological Chemistry,* vol. xv, No. 3, Sept., 1913.

The other method, also suggested by Dr. Marshall, is to draw 5 cubic centimeters of blood from a vein with a hypodermatic needle, into a five cubic centimeter pipette, and immediately transfer the specimen to a test-tube, containing 1 to 2 cubic centimeters of one percent sodium oxalate solution. To this is added 25 milligrammes of urease, previously dissolved in 5 cubic centimeters of water. This mixture is allowed to stand until the urea of the blood is decomposed; at ordinary room temperature, one-half hour is usually sufficient. It is better, however, to place the test-tube in a beaker of water at 30° to 40° C. for one-half hour. After the urea has been changed, the contents and sufficient washings of the tube are transferred to a cylinder. The ammonia is then removed by a current of air, collected in fiftieth-normal hydrochloric acid and titrated with fiftieth-normal sodium hydroxide.

CALCULATING UREA CONTENT.

As the purpose in using urease is to convert the urea present in a specimen into an easily estimated substance—ammonium carbonate—and as the amount of this salt produced from this source by the enzyme is indicated by the increased alkalinity of the specimen to methyl orange, it is obvious that the quantity of standard hydrochloric acid required to exactly neutralize the contents of the flask containing urease, less the quantity required for the control specimen, corresponds to the ammonium carbonate formed by the conversion of the urea originally present in the specimen.

By the equation appearing at the beginning of this paper, it will be readily seen that 60 grammes of urea would be converted, by urease, into 96 grammes of ammonium carbonate, which amount would require 72 grammes of standard hydrochloric acid to neutralize it.

As this quantity (72 grammes) of hydrochloric acid is contained in twenty thousand cubic centimeters (20,000 Cc.) of decinormal $(\frac{N}{10})$ hydrochloric acid solution and is equivalent to 60 grammes of urea, as represented by 96 grammes of ammonium carbonate, it follows that one twenty-thousandth (1/20,000) of this quantity, or one cubic centimeter (1 Cc.), of decinormal hydrochloric acid would be the equivalent of one twenty-thousandth of 60 grammes—three milligrammes ($60 \div 20{,}000 = .003$); therefore, each cubic centimeter of decinormal hydrochloric acid required to neutralize an enzyme treated specimen that is in excess of the number of cubic centimeters required to neutralize the control specimen represents 3 milligrammes of urea, and, as the 5 Cc. specimen is the one two-hundredth part of a liter, it will be only necessary to multiply the number of cubic centimeters of the decinormal hydrochloric acid solution, in excess of the control's requirements, by the factor, $.6(.003 \times 200. - .6)$, to ascertain the urea per liter, when estimating the daily output.

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