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## The preparation of soy bean urease in solid form and its use in urea determination.

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In the course of work in which we have been utilizing the soy bean urease, recently introduced by Marshall into analytical chemistry, we have found it advantageous to prepare and keep the enzyme in solid form. The fine-ground beans are covered with 5 parts of water and allowed to stand an hour with occasional shaking. The extract is then pressed through cheese cloth, and either filtered or centrifuged. The enzyme in solid form is obtained from the extract by either: (1) Precipitation, by pouring the extract into at least 10 volumes of acetone; (2) concentration of the extract to dryness at room temperature at a pressure less than 1 mm. The dry powder obtained by either method can be dissolved in a few seconds in 10 parts of water, and the solution obtained is so active that it permits very rapid analyses. *Urine* (human) is diluted tenfold. Three c.c. (= 0.3 c.c. urine) are mixed in a 100 c.c. test tube with 2 c.c. of an 8 per cent. urease solution. A drop of caprylic alcohol (to prevent subsequent foaming) is added, and the mixture allowed to stand ten minutes at room temperature (18° or over), three minutes at 40°, or two minutes at 50°. The ammonia is then drawn off by ten minutes' aeration (Folin's method) into 20 c.c. of N/50HCl. The stoppers are placed in the tubes as soon as the urease is added, and the aeration run a half minute before opening the tube to add the alkali (4 grams solid $K_2CO_3$). *Blood:* 5 c.c. of freshly drawn blood are mixed with 1 c.c. of 5 per cent. potassium citrate, 1 c.c. of 8 per cent. urease, and 4 drops of caprylic alcohol. Remainder as with urines. If Folin's colorimetric method for determining the ammonia is used, 1 c.c. of blood suffices. *Aqueous tissue extracts* are brought to a volume of 0.5 to 1 c.c. per gram of tissue, and 5 c.c. portions are treated as described for blood, except that only 1 drop of caprylic alcohol is needed. In case the extracts have been acidified with acetic acid to coagulate proteins, 1 c.c. of 15 per

cent. $K_2HPO_4$ is added before the urease. *Time law of urease action:* The velocity of urea decomposition is expressed by the isotherm,

$$t = \frac{1}{e}\left(x + a \log \frac{b}{b + x}\right),$$

where $t$ is the time of reaction, $e$ the enzyme concentration, and $x$ the concentration of ammonia formed. The derivation of the equation will be given later.

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## A method for the estimation of sugar in small quantities of blood.

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The red color obtained by heating a dextrose solution with picric acid and sodium carbonate is employed as the basis of the present method for the determination of blood sugar, the reaction being so delicate that it is possible to determine the dextrose in as little as 0.5 c.c. of blood. Following is the method in detail as ordinarily used by us.

Two c.c. of blood are drawn from a vein through a hypodermic needle into an Ostwald pipette, a little potassium oxalate in the tip of the pipette preventing clotting. The blood is discharged immediately into a 25 c.c. volumetric flask containing 10 c.c. of N/100 acetic acid previously heated in a boiling water bath. The pipette is rinsed once with distilled water. The flask is replaced in the boiling water bath and shaken occasionally for five minutes. After cooling, 1 c.c. of 5 per cent. dialyzed iron (Merck) is added to precipitate any protein still in solution. Distilled water is added to the mark, the contents of the flask are filtered, and an aliquot of the clear filtrate (10 c.c. or 15 c.c.) is measured into a large Jena test tube (200 × 22 mm.) and evaporated to 1 c.c. or below (but not to dryness) over a direct flame, two glass beads being used to prevent bumping. Two c.c. of saturated picric acid solution and 3 c.c. of 20 per cent. sodium carbonate are added