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An Error in the Urease Method for the Determination of Urea.*

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Estimates of the urea concentration of the blood, muscle and liver tissue of rats were carried out in connection with a study of the effects of high protein diets. The urease method was used. A typical result is given in Table I.

TABLE I.
Urea Concentration in Blood, Muscle and Liver.

Diet	Blood	Muscle	Liver
	mg. per 100 gm.	mg. per 100 gm.	mg. per 100 gm.
Carbohydrate and fat	24	26	207
Protein 74%	52	44	258

The high concentration of urea shown here for the rat's liver is at variance with the observation of Marshall[1] who found an approximate equality of urea concentration in the blood, muscle and liver of other animals. In obtaining the results shown in Table I the rats were anesthetized with ether, and bled to death by cutting the abdominal aorta. The tissues were removed immediately, weighed, ground in a mortar and quantitatively transferred with water to large aeration tubes. The method used has been described in detail elsewhere.[2] The only changes made were those which were necessitated by the fact that the tissues and blood were made up with water to a volume of 30 cc. This method is simply a special adaptation of the aeration method described by Marshall.[1] The urea is calculated from the amount of additional ammonia formed after incubation of the tissue with the filtrate from a suspension in water of freshly ground jack beans.

When the liver was heated before adding the jack bean filtrate an entirely different result was obtained, the previous high level of urea concentration in the liver was no longer found, and the estimates showed an approximate equality of concentration in blood, muscle, and liver, in accordance with the conclusion reached by Marshall. (Table II.)

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TABLE II.
The Effect of Heat on the Urea Concentration of the Liver as determined by the Urease Method.

Liver ground up in boiling water.	Liver ground up in cold water.
mg. per 100 gm.	mg. per 100 gm.
23	296
18	224

A linear relation is attained between the quantities of urea produced and the quantities of "urease" used when the ratio between liver and urease is neither too small nor too large. Fig. 1 shows the result of an experiment in which the liver was held constant at 2.26 gm. and the urease was varied from 0.2 to 1.0 gm. The mixtures were incubated for 2 hours at a temperature of 37° C. The reaction was maintained at pH 7.2 by phosphate.

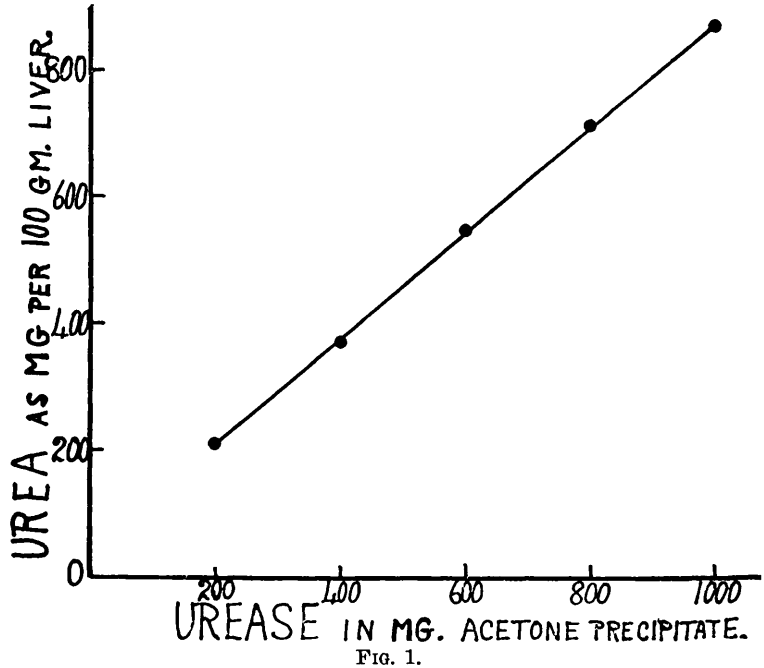

FIG. 1.

In ox blood there is a heat labile agency, confined to the red blood cells, which is capable of forming urea from the acetone precipitate of a water solution of jack beans. The results given in Table III show that the concentration of urea in human blood may be varied by adding different amounts of this acetone treated "urease."

With blood the additional urea produced by increasing the amount of urease is less than when liver is used, but the figures show that the concentration of urea is from 2 to 3 times greater when large

TABLE III.
Increase in Concentration of Urea in Human Blood with increase in amount of Urease added.
Blood and Urease incubated for 60 min. at 38° C.

Subject	Blood	Urease	Concentration of Urea in Blood
	cc.		mg. per 100 cc.
T. A.	2	2 cc. 10% Jack Bean filtrate	33.
,,	,,	100 mg. acetone precipitate	51.
,,	,,	500 ,, ,,	73.
,,	,,	1000 ,, ,,	77.
L. J. P.	2	2 cc. 10% Jack Bean filtrate	39.
,,	,,	100 mg. acetone precipitate	67.
,,	,,	500 ,, ,,	120.
,,	,,	1000 ,, ,,	128.
W. S. P.	2	20 mg. acetone precipitate	22.
,,	,,	200 ,, ,,	34.
,,	,,	400 ,, ,,	34.
,,	,,	800 ,, ,,	39.
,,	,,	1200 ,, ,,	40.
E. B.	2	20 mg. acetone precipitate	19.
,,	,,	200 ,, ,,	29.
,,	,,	400 ,, ,,	30.
,,	,,	800 ,, ,,	35.
,,	,,	1200 ,, ,,	35.

than when small quantities of urease are added. This confirms the observations of Miss Behre.[3] Her investigations into the origin of the additional urea led her to the belief that an enzyme present in the soy or jack bean may have formed urea from some unknown constituent of the red blood cells. This hypothesis has not yet been investigated with respect to the blood but it is inapplicable as an explanation of the facts reported here in regard to the liver. For even after "urease" is heated to a temperature at which all known enzymes are inactivated, it still acts as a source of urea formation when incubated with liver. The contrary hypothesis, namely, that the liver contains the enzyme, and that "urease" is the substrate, has therefore been adopted.

No facts have as yet been encountered which would negative the conception that the additional urea is derived from the action of arginase in the liver on arginine in the jack bean. In view of the mode of preparation of some of the bean extracts it is unlikely that this arginine can be free. But proteolytic enzymes in the liver may set arginine free, or it is even conceivable that one of the proteins of the jack bean has arginine so situated in its structure that urea may be formed from it under the action of arginase. An attempt is being made to study these and other related questions.

[1] Marshall and Davis, *J. Biol. Chem.*, 1914, xviii, 52.

[2] Addis, *J. Lab. and Clin. Med.*, 1924, x, 402.

[3] Behre, *J. Biol. Chem.*, 1923, lvi, 395.