present in the standard thyroxin solution exhibited marked indications of metamorphosis which were comparable to those produced by the standard thyroxin solution. The posterior limbs of the animals immersed in this solution of diiodotyrosine grew to an average length of 2.04 mm. as compared with 2.34 mm. in the animals treated with thyroxin. Potassium iodide, in the concentrations used, showed no thyroxin-like properties. That this compound lacks this characteristic, however, is not a necessary conclusion for, as Swingle (1919) has indicated, still higher concentrations over greater periods of time may produce the desired effect. Experiments which may yield quantitative data on this question are now being planned in this laboratory. On the basis of the data now on hand, however, it may be said that iodine in diiodotyrosine is probably more than 5 times as active as that in potassium iodide.

Summary. Iodine as it occurs in thyroxin is over 300 times as effective in inducing precocious amphibian metamorphosis as that occurring in diiodotyrosine. The iodine in diiodotyrosine, however, is far more active than that included in potassium iodide.

9227 P

Utilization of Ketone Bodies by the Tissues in Ketosis.

RICHARD H. BARNES AND D. R. DRURY.

From The Scripps Metabolic Clinic, La Jolla, California, and Department of Physiology, University of Southern California School of Medicine.

The work of Chaikoff and Soskin[1] and Mirsky[2] has shown very definitely that the ketone bodies are produced only in the liver. Are the ketone bodies so formed utilized by the tissues in nutritional conditions giving rise to ketosis? This note deals with this question.

A male human was kept on a ketogenic diet for several days. After a definite ketonuria had developed (5 gm. ketone bodies per day), food was withheld during the morning and blood ketone concentrations at 7 A. M. and 11 A. M. were determined (the first blood 12 hours after the previous meal). The 7 A. M. blood contained 26.6 mg. ketones %, the 11 A. M. sample 10.3. The experiment was repeated on 2 other mornings with the following results:

1. Blood ketones 7:20 A. M. 26.4, 11 A. M. 9.5.

1 Chaikoff, I. L., and Soskin, S., *Am. J. Physiol.*, 1928, **87**, 58.

2 Mirsky, A., *Am. J. Physiol.*, 1936, **116**, 110.

2. Blood ketones 7:50 A. M. 35.9, 12 Noon 8.8.

The results of Chaikoff and Soskin indicate that the ketone bodies diffuse very readily throughout the tissues of the body, so that we may assume that the subject (wt. 70 kilos) had at least 50 liters of water in his body carrying the ketones bodies in the same concentration as the blood. On this assumption the amount of ketones disappearing from the body during the mornings cited must have been (difference in concentrations of the blood ketones X 50 liters), 8.15 gm., 8.45 gm., 13.5 gm. The ketone output in the urines of these periods was 472 mg., 278 mg., 538 mg. This suggests a very considerable utilization of ketones by the tissues under a definitely ketogenic condition—averaging 2.44 gm. per hour, and does not include what might have been produced concurrently by the liver. All the foregoing estimations were carried out by the methods of Van Slyke (urine) and Van Slyke and Fitz (blood).

To obtain data bearing on the total tissue utilization arterio-venous ketone-body differences were determined in various conditions which give rise to definite ketosis. The estimations were carried out by the Barnes method.[3] The figures indicate the total ketone bodies expressed as mg. acetone per 100 cc. blood.

TABLE I.

Species	Condition	Arterial Blood	Venous Blood
Man	Ketogenic diet 2 days, fasting 20 hr.	3.3	2.4
,,	,, ,, ,, ,, ,, ,,	4.4	2.7
,,	,, ,, 3 ,, ,, ,, ,,	17.3	14.1
Dog	2 days fast and phloridzin	3.2	1.9 (Femoral) 2.5 (Jugular)
,,	4 ,, ,, ,, ,,	19.3	17.7 (Femoral) 17.7 (Jugular)
,,	,, ,, ,, ,, ,,	14.0	11.8
,,	Depancreatized without insulin 30 hr.	3.5	2.9
,,	,, ,, ,, ,, ,,	3.7	3.5
Rabbit	Fasting and injection of ketogenic extract of ant. pituitary	7.2	6.5

The differences are significant, since for man an arterio-venous difference of 1 mg. % and a tissue blood flow of 5 liters per min. would indicate a utilization of 3 gm. ketones per hour. This is of the same order of magnitude as the figure given above. The oxidation of such an amount would give rise to considerable energy and would represent an appreciable fraction of the total metabolism. We may conclude then that the mechanism: conversion of fats to ketones by the liver and oxidation of this by the tissues, may account for an

[3] Barnes, R. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 352.

important amount of the metabolism of individuals having "ketosis". It follows that this mechanism of fat utilization could operate when the body is oxidizing fat without showing ketosis.

9228 P

A Method for Determination of Blood Acetone Bodies.

RICHARD H. BARNES. (Introduced by Eaton M. MacKay.)
From the Scripps Metabolic Clinic, La Jolla, California.

A satisfactory method for the determination of the acetone bodies in blood at low levels or suitable for use with small volumes of blood has not hitherto been available. An attempt has been made to devise a method which combines the sensitivity of the iodine titration (Hubbard's Method) with the specificity of the Denigè's-Van Slyke method. In this method the ketone bodies are precipitated as in Van Slyke's method, this precipitate is freed from contaminating material by washing, it is dissolved in acid and is distilled with heat into alkaline iodine which can be titrated with thiosulfate. Oxidation of the ketones is carried out in a large centrifuge tube with a small volume of liquid, which allows the acetone precipitate to form with very small quantities of acetone. Barium chloride is added and the fine barium sulfate precipitate helps to prevent the loss of the acetone mercuric sulfate precipitate when decanting.

Briefly the procedure is as follows: 2 cc. of oxalated blood is lyzed with 14 cc. water in a 50 cc. centrifuge tube and 14 cc. of mercuric sulfate solution (as in the Van Slyke method[1]) added with shaking. After standing an hour this is centrifuged and the supernatant liquid filtered. Ten cc. of filtrate* is placed in a special centrifuge tube with a ground glass joint and 4 cc. of a solution added which contains per 100 cc.: 70 cc. of the above mentioned mercuric sulfate solution, 20 cc. of 50% sulfuric acid, and 10 cc. of water. Connected by the glass joint to a reflux condenser this mixture is boiled for 90 minutes. As soon as boiling has commenced 0.5 cc. of 5% potassium dichromate is added. The tube is then cooled, 3-4 drops of 10% barium chloride is added and then centrifuged at high speed for 10 minutes and the supernatant liquid carefully poured off.

[1] Peters and Van Slyke, *Quantitative Clinical Chemistry*, 625.

* Filtrates prepared in this manner from diabetic blood must be treated further for the high sugar present.