10770

Acetone Fractionation of Blood and Urinary Iodine.

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Correlation of the forms in which iodine exists in the blood with those found in the urine is of significance in determining the fate of thyroxine in the human body. Barkan[1] separated the "organic" iodine of the blood and urine from the "inorganic" by precipitation with silver nitrate. He reports that from 20 to 70% of the total iodine in normal urine is organically bound. Foster and Gutman[2] fed diiodotyrosine to rabbits and found 10% of the urinary iodine as inorganic iodide, 60% as unchanged diiodotyrosine, 18% as 3,5-diiodo-4-hydroxyphenyllactic acid, and 12% as iodine lost in the separation. Eufinger and Schulte[3] employed an 80% acetone solution for the separation of "organic" and "inorganic" iodine in the blood.

We have employed a 75% acetone solution in fractionating both the blood and urinary iodine. The urine specimens were collected in 3-day (72-hour) periods. The blood was drawn and the B.M.R. determined on the second day of each period. The periods studied were: I. Normal, nonconstant regimen with no iodine medication; II. Normal, nonconstant regimen with 10 mg of iodine as KI given daily for 7 days—the last 3 days being the period of study; III. Normal, nonconstant regimen with 3 grains of desiccated thyroid given daily for 18 days—period of study covering the 18th, 19th and 20th days after starting the thyroid medication. All medication was given orally. The subject was a normal male.

One hundred ml of urine is treated with 300 ml of 99.5% iodine-free acetone. The mixture is shaken well and allowed to stand over night. The precipitate is then filtered off and washed well with acetone. The filtrate and washings are analyzed together for iodine and termed the acetone-soluble fraction. The precipitate is then washed with 500 ml of double-distilled water, which is analyzed for iodine and termed the acetone-insoluble but water-soluble fraction. The remaining precipitate is then analyzed for iodine and termed the acetone and water-insoluble fraction.

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1 Barkan, G., *Arch. f. Exp. Path. und Pharmakol.*, 1928, **138**, 160.

2 Foster, G. L., and Gutman, A. B., *J. Biol. Chem.*, 1930, **87**, 289.

3 Eufinger, H., and Schulte, W., *Arch. f. Gynak.*, 1933, **152**, 479.

TABLE I.
Urinary Data for the Three Periods of Study (3 days per period).

	Period I No Medication B.M.R. % —8			Period II 10 mg Iodine as KI B.M.R. % —8			Period III 3 grs Desiccated Thyroid B.M.R. % +5		
Day	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Total urinary volume (ml)	950.0	690.0	860.0	750.0	800.0	675.0	1640.0	1345.0	1340.0
Total iodine output (gamma*)	231.0	97.3	131.0	730.0	542.0	682.0	445.0	291.0	192.0
" " (gamma %)	24.3	14.1	15.2	97.3	67.7	101.0	27.1	21.6	14.3
Acetone-soluble iodine (gamma %)	24.1	13.5	15.6	96.5	66.9	100.2	26.3	20.5	13.1
Acetone-insoluble-water-soluble iodine (gamma %)	0.20	0.42	0.10	0.67	0.61	0.58	0.31	0.21	0.35
Acetone- and water-insoluble iodine (gamma %)	0.0	0.0	0.0	0.15	0.11	0.16	0.0	0.0	0.0

* 1 gamma = 1 microgram = .001 mg.

TABLE II.
Blood Iodine Data for the Three Periods of Study.
(One blood analysis per period—blood drawn on 2nd day of period.)

	Period I No Medication B.M.R. % —8	Period II 10 mg Iodine as KI B.M.R. % —8	Period III 3 grs Desiccated Thyroid B.M.R. % + 5
Acetone-soluble iodine (gamma %)	1.50	5.33	1.94
Acetone-insoluble-water-soluble iodine (gamma %)	0.93	1.10	2.60
Acetone- and water-insoluble iodine (gamma %)	1.20	1.10	1.36
Total iodine (gamma %—calculated from fractions)	3.63	7.53	5.90

The above procedure was used in the blood fractionation except that 50 ml of fresh, untreated blood and 150 ml of acetone were employed.

The Matthews, Curtis and Brode[4] modification of the Leipert procedure was employed in determining the iodine content of the 3 fractions.

The results of the urine analyses for the 3 periods studied are given in Table I. It will be seen from this table that all of the urinary iodine is acetone-soluble regardless of the form of iodine ingested. The results of the blood analyses for the 3 periods of study are given in Table II. From the data presented in this table a definite variation in the iodine content of the fractions will be noted. This appears to be due to the form of iodine ingested. From these data it would appear that the acetone-soluble fraction contains that portion of the blood iodine eventually excreted in the urine and that the other 2 blood fractions contain the iodine that is not excreted in that form by the kidneys. These two fractions apparently contain iodine in a different form than the urinary iodine. It is not to be assumed, however, that all of the iodine excreted in the urine or found in the acetone-soluble fraction of the blood exists in a form that cannot be utilized by the body.

It is of interest to note the dominance of the acetone-soluble fraction of iodine in the normal blood as well as after potassium iodide medication, whereas after thyroid medication the acetone-soluble fraction falls, while the acetone-insoluble but water-soluble fraction appears to increase. The significance of the variation of these blood iodine fractions is as yet unknown.

Summary. 1. All of the urinary iodine appears to be acetone-soluble. 2. There is an acetone-soluble; and acetone-insoluble but water-soluble; and an acetone- and water-insoluble form or forms of iodine in the blood. 3. The quantitative relationship between the iodine fractions of the blood varies with the form of iodine ingested. 4. The acetone-soluble fraction of the blood iodine may possibly contain the iodine compounds which will later be excreted in the urine. 5. The true significance of these fractions is as yet unknown.

[4] Matthews, Norman L., Curtis, George M., and Brode, Wallace R., *Ind. and Eng. Chem.*, Anal. Ed., 1938, **10**, 612.