AN EXTRACTION-PHOTOMETRIC METHOD OF MICRODETERMINATION OF IODINE IN FLUIDS AND TISSUES AFTER ADMINISTRATION OF IODINE-CONTAINING COMPOUNDS TO MAN AND ANIMALS

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UDC 616-008.921.5-074

A micromethod is suggested for determining the iodine concentration in the urine, blood, and tissues after administration of iodine-containing compounds. The method, which is based on extraction of the blue-green salt-like complex formed by the polyhalide ion I_2Br^- with the cation of brilliant green, with toluene is distinguished by its specificity, great simplicity, and accuracy. The error of determination does not exceed ±2.5%.

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Extraction-photometric methods suggested for the determination of certain trace elements [1, 2, 4-8] were based on the principle of extraction of salt-like compounds formed by the cation of brilliant green with anions containing the investigated element ($SbCl_6^-$, PCl_4^-, I_2Cl^-, I_2Br^-). The extraction is carried out with benzene and its homologs. An advantage of these methods is that extraction is carried out as a single procedure and direct determination of iodine and other trace elements is possible without preliminary combustion of the material or treatment of it with concentrated acids [3, 9-11].

Reagents: 1) 10% NaCl solution, 2) 0.5% freshly prepared solution of sodium nitrite, 3) 20% solution of sodium bromide, 4) 5N H_2SO_4, 5) 1% acetic acid solution, 6) toluene, 7) 0.5% solution of brilliant green ("indicator for microscopy" grade) in an alcoholic mixture consisting of 25 ml 96° alcohol and 75 ml water.

Estimation of iodides in the urine. With a micropipet, 0.1 ml of the urine to be tested is measured into a 50-ml Kjeldahl flask containing 5 ml 20% NaCl; 0.25 ml of the nitrite solution and the same volume of solutions of sodium bromide and brilliant green are added. The contents of the flasks are shaken, and 5 ml toluene (from a buret) and 1 ml 5N H_2SO_4 are added, the mixture then being shaken vigorously for 2 min. The colored layer of toluene formed on standing is transferred into a test tube, and from it into the 10-ml cuvette of a horizontal FM-56 photometer. The intensity of the color is measured against the red scale of the drum, using an M-61 filter. Toluene obtained by identical treatment of the urine in another flask, but without addition of nitrite, is poured into the second cuvette for compensation.

For calculation the formula x = (a-b):0.097 was used, in which x is the unknown concentration of iodine in μg/0.1 ml urine, *a* the reading on the red (logarithmic) scale of the drum, and b the absolute error depending on the iodine concentration in the reagent. The figure 0.097 corresponds to extinction of 1 μg iodine using a 10-ml cuvette. If it is desired to express the results of the estimation not as iodine, but as potassium iodide, a divisor of 0.074 is used. To determine the absolute error, toluene obtained by treatment of all reagents in the manner described above, but without the addition of urine, is poured into one cuvette, and the toluene extract formed after analogous treatment of all reagents except nitrite into the second cuvette. The value obtained by calculation corresponds to the iodine concentration in μg/0.1 ml urine or to the iodine content in mg/100 ml.

If the reading on the red scale exceeds 1.00, the urine is first diluted 5 or even 10 times. If the readings are less than 0.05, instead of 0.1 ml, from 0.2 to 0.5 ml of undiluted urine is measured out.

Estimation of iodides in the blood and tissues. To determine iodine in the blood after administration of iodides or other iodine-containing compounds, the proteins are first precipitated. To 1 ml water poured into a graduated centrifuge tube, 0.2 ml of blood taken from the finger or of preliminarily defibrinated blood is added. Next, from 6 to 8 ml of 20% NaCl solution is added to the tube; the liquid is acidified with 0.2 ml

Department of Physiology and Biochemistry of Man and Animals, Samarkand State University (Presented by Academician of the AMN SSSR S. R. Mardashev). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 4, pp. 124-125, April, 1969. Original article submitted January 23, 1968.

TABLE 1. Determination of Quantity of Iodide Added to Urine and Blood

Volume of urine and blood used in experiment (in ml)		KI added (in μg)	KI content determined experimentally (in μg)	Error of determination (in %)	Volume of urine and blood used in experiment (in ml)		KI added (in μg)	KI content determined experimentally (in μg)	Error of determination (in %)
	0.6	1.80	1.76	−1.1		0.2	4.82	4.82	0
	0.1	0.55	0.54	1.8		0.2	1.67	1.68	+0.6
Urine	0.1	23.50	23.70	+0.8	Blood	0.1	5.5	5.5	0
	0.1	17.30	17.35	+0.3		0.1	25.0	25.2	+0.8
	0.1	9.40	9.45	+0.5		0.1	16.4	16.0	−2.5

of 1% acetic acid, the contents are thoroughly mixed with a thin glass rod, and they are immersed together with the rod for 5 min in a boiling water bath. After this time the tube is removed and cooled by immersing it in a small jar filled with cold water. To bring the volume of liquid in the tube up to 10 ml, sodium chloride solution is added drop to it, holding the rod in the hand, until the lower border of the meniscus reaches the division marked 10. The contents of the tube are then vigorously stirred with the same rod, after which the liquid is filtered through a corrugated filter 6 cm in diameter. The filtrate must be absolutely colorless and transparent; 5 ml of it is transferred into a 50-ml Kjeldahl flask and treated with nitrite, bromide, an aqueous-alcoholic solution of brilliant green, toluine, and sulfuric acid just as described for analysis of urine.

For the calculation, the extinction of a control specimen, set up by the same formula as given above but using blood from a person not receiving iodide, is subtracted from the reading on the red scale of the drum.

The iodine concentration in the tissues is determined in the same way. A weighed sample of tissue, measuring about 500 mg, is triturated in a small porcelain mortar with a small volume of finely powdered and washed glass to obtain a homogeneous mass which is transferred quantitatively into a graduated centrifuge tube, washing out the contents of the mortar with 20% NaCl. An approximately equal second weighed sample, treated in the same way but without the addition of nitrite to the filtrate, is used for compensation.

The method possesses high reproducibility. Results of the determinations after addition of different quantities of iodide to urine and blood are given in Table 1. The error of determination does not exceed ±2.5%.

The method as developed is of practical importance because the dynamics of accumulation and elimination of iodine after administration of iodine-containing compounds has not hitherto received adequate study.

Instead of the FM-56 photometer, various types of photoelectric colorimeters (FEK-56, FEK-N, etc.) can be used for the determination.

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