teins present failed to effect complete destruction of the toxicity. Treatment of the extract with both boiled and unboiled solutions of pancreatin discloses no destruction of the toxic substance by enzyme action.

11707

Modified Method for Determination of Certain Organic Iodine Compounds, Inorganic Iodide in Plasma and Urine.*

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A method for the determination of diodrast and inorganic iodide iodine in blood and urine was presented previously.[1] The principles involved were acid permanganate digestion of the sample with oxidation of iodine to iodate, nitrite reduction of the permanganate, destruction of excess nitrite with urea, and titration of the iodate with thiosulphate in an excess of potassium iodide. This procedure could be carried out either by hand heating of the individual tubes over a micro burner or by heating a number of tubes in a boiling water bath. The digestion of individual samples by hand yields accurate results but requires the constant attention of the analyst. The water bath digestion, however, involves some error, particularly in the digestion of plasma filtrates at high iodine levels, and it is sometimes more difficult to remove all of the permanganate because some manganese dioxide adheres firmly to the walls of the tubes. It is shown here that with an alkaline permanganate digestion these difficulties with the water bath heating are eliminated.

With an alkaline digestion more permanganate must be used, and a longer heating period allowed than with the acid digestion. In order to keep down the volume of the digest at time of nitrite treatment and at subsequent titration, preliminary evaporation of filtrate samples is carried out.

Procedure. Reagents: 13% trichloracetic acid (A.R.), 72% sodium hydroxide (A.R.), approximately 0.4 M potassium permanganate, 4 N sulfuric acid, 1.0 M sodium nitrite, 5 M urea, granulated potassium iodide, 1% starch, 0.0005 N sodium thiosulfate or 0.0025 N sodium thiosulfate.

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1 White, H. L., and Rolf, D., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 1.

Apparatus: circular test-tube rack, water bath, 18x150 mm test tubes, dropping bottles, burette calibrated to 0.01 cc or 0.05 cc.

In preparing the potassium permanganate make a 7% solution, boil for 5 to 10 minutes and let stand for at least 24 hours. After standing, filter through asbestos or sintered glass and standardize. This standardization need not be extremely accurate, since it is only necessary to establish that the permanganate is at least 0.4 M. If it is more than 0.4 M, use the quantities given in the procedure but if it is less than 0.4 M add a correspondingly greater amount.

PLASMA. Precipitation. Pipette 1 volume of plasma into a small Erlenmeyer flask, add 7 volumes of water and 2 of 13% trichloracetic acid, shake and filter after about 5 minutes.

Digestion. Pipette 3 cc of filtrate into an 18x150 mm test tube, place the tube in a rack and evaporate the filtrate to a volume of about 1.5 cc by suspending the rack in a boiling water bath for 30 minutes. If 2 cc, or 4 or 5 cc, of filtrate are used, evaporate 10 minutes and 40 minutes, respectively. If 1 cc of filtrate is used add 0.5 cc of water and do not evaporate. With an iodine level between 1 and 4 mg%, 3 cc of filtrate will be sufficient; below 1 mg%, 4 or 5 cc is advisable. Above 5 mg%, 2 cc or 1 cc may be used, and if the level is above 20 mg% it is necessary to dilute the filtrate. After evaporation remove the rack from the bath, add 0.1 cc of 72% sodium hydroxide, 1 cc of 0.4 M permanganate, shake the individual tubes gently, place the rack in the boiling water bath for 15 minutes and shake the tubes every 4 to 5 minutes. The tubes can be easily shaken if one uses a piece of gauze to protect the hands. Gummed labels identify the samples. After 15 minutes remove the rack from the bath, add 0.8 cc 4 N sulfuric acid and mix by shaking. Treating each tube individually, add 1 M sodium nitrite from a dropping bottle until the permanganate is almost gone; finish the treatment with 0.5 M sodium nitrite, adding one full drop in excess. Shake the tubes after the addition of each drop of nitrite; wash the walls of the tubes by tilting them so that all traces of permanganate are removed. Place the rack in the bath for 2 minutes; during this interval frequently lift each tube from the bath and shake. At the end of 2 minutes remove the rack from the bath, add 4 drops of 5 M urea from a dropping bottle so that the drops wash down the walls of the tubes. Replace the rack in the bath for 1 minute. After about 30 seconds, lift each tube from the rack and shake. At the end of the minute, remove the rack from the bath and wash the walls of the tubes with the hot digest, tilting the tubes so that the solution reaches any portion which may have been touched by nitrite. Place the rack in the bath for about 1 minute

longer, repeating the procedure outlined in the preceding two sentences. One can easily handle 6 to 8 samples at one time but it is not advisable to treat more than 10. The digestion and subsequent treatment of 10 tubes after evaporation has been completed will take about 25 to 30 minutes.

Titration. Titrate under artificial light with 0.0005 N thiosulphate, in an excess (few tenths of a milligram) of potassium iodide.† No measurable blank is obtained on water. A blank titration on 3 cc of normal plasma filtrate will be from 0.003 to 0.01 cc; when this is disregarded practically 100% recoveries are obtained, which indicates that a very slight loss of iodine is thereby compensated. Recoveries of the iodine of diodrast added to plasma filtrate are shown in Table I.

URINE. Digestion. Dilute the urine so that 1 cc contains from 5 to 500 μg of iodine. Place 1 cc of diluted urine plus about 0.5 cc of water in a test tube and treat exactly as outlined for the filtrates except that 0.6 cc of permanganate is used.

Titration. If the sample contains 60 μg or more of iodine, titration can be carried out in the daylight, using a burette calibrated to 0.05 cc and 0.0025 N thiosulfate. Employing this titration the tubes need only to be cooled to room temperature. More potassium iodide (1

TABLE I.
Percentage Recovery of Diodrast Added to Normal Plasma Filtrate.
A 1 mg % filtrate means that 1 mg of diodrast iodine has been added to 1000 cc of a 1 to 10 filtrate of normal plasma.

1 mg % filtrate			2 mg % filtrate	4 mg % filtrate		10 mg % filtrate	
3 cc	4 cc	5 cc	3 cc	2 cc	3 cc	1 cc	2 cc
101.0	99.3	100.2	99.3	98.6	100.5	99.4	97.6
95.8	98.7	105.8	99.1	98.6	98.4	99.1	99.2
102.0		102.5	99.8	99.8	99.8	99.4	99.6
99.0		97.5	100.5	98.2	100.6	100.0	99.0
102.7		97.5	102.2	97.6	99.1	100.0	
97.3			99.0	97.5	98.1		
			97.3	97.6	98.5		
			99.4	98.8			
				98.5			
				97.5			

† It has come to our attention that the direction in our original communication to add "a few crystals" of potassium iodide has sometimes been misinterpreted. We use a few granules of "granulated" potassium iodide, totaling about 0.5 mg. If one takes a few crystals of "crystalline" potassium iodide he will have many times the optimum amount, with the result that significant amounts of iodine will be liberated, leading to an erroneously high titration and inability to reach a definite endpoint. Furthermore, the direction to "titrate in an artificially lighted room" means that the room should be reasonably dark in the absence of the artificial light.

TABLE II.
Percentage Recovery of Iodine from Aqueous Solutions.

	Amount of iodine in sample						
	5 μg	7.5 μg	10 μg	100 μg	200 μg	300 μg	500 μg
Aqueous solution of diodrast	99.9	99.9	98.7	101.0	99.4	99.4	99.6
	101.9	100.0	100.4	100.0	99.3	100.2	100.5
	99.2	98.9	100.0	100.0	99.3	99.4	100.6
	100.0	99.9	99.4				100.3
	101.0	101.0	99.4				
	100.0	99.6	99.5				
	100.4		99.2				
	99.4		99.9				
	99.8		100.0				
	99.4		99.5				
	99.8						
Aqueous solution of diodrast compound				100.5			
				100.2			
				100.5			
Aqueous solution of KI				101.5			
				100.5			
				101.5			

to 10 mg) is needed with 50 to 500 μg iodine in the sample than is needed for the "micro" titration. If the sample contains less than 25 μg of iodine, the titration should be carried out under artificial light at 10° C or lower, using 0.0005 N thiosulphate and a burette graduated to 0.01 cc.[1]

This same procedure has been used for the determination of water solutions of potassium iodide and diodrast compound, recovery being complete (See Table II). A few determinations on water solutions of hippuran have yielded 97 to 98% recovery when the alkaline digestion is carried on for 75 minutes. In this case it is necessary to use 1.2 cc of 0.4 M permanganate with periodic addition of distilled water to keep the volume up to 1.5 to 2 cc, and to add 1 cc of 4 N sulfuric acid before the addition of sodium nitrite. Some cloudiness is observed in hippuran digests so treated; this no doubt somewhat increases the error of the titration but has not prevented recoveries of the accuracy mentioned.

It was pointed out before[1] that some diodrast is carried down during the plasma precipitation, but that the percentage recovery from the filtrate of known amounts of diodrast added to the plasma before precipitation is constant on separate precipitations of the same plasma, even at various iodine levels (2.5 to 30 mg I per 100 cc). It was further shown that this percentage recovery does not vary greatly with different normal subjects. The previous average

figures for the percentage of diodrast coming through in plasma filtrates of normal humans and dogs were 84.6 and 84.7%, respectively. More recent findings place these averages at 86.9% for humans and 85.9% for dogs, with average deviations of 1.7% and 1.4% respectively. This figure can be established for each subject when the highest accuracy is desired.

CALCULATIONS. For plasma filtrate,

$$\text{cc 0.0005 N thiosulphate} \times \frac{10.6}{1000} \times \text{dilution factor} \times \frac{100}{\%\ \text{coming through}} = \text{mg iodine per 100 cc plasma.}$$

Example, using 3 cc of plasma filtrate,

$$0.530 \times \frac{10.6}{1000} \times \frac{1000}{3} \times \frac{100}{86.9} = 2.15 \text{ mg iodine per 100 cc plasma.}$$

For urine,

(1) using 0.0005 N thiosulphate,

$$\text{cc thiosulphate} \times \frac{10.6}{1000} \times \text{dilution factor} \times 100 = \text{mg iodine per 100 cc urine.}$$

(2) using 0.0025 N thiosulphate,

$$\text{cc thiosulphate} \times \frac{53}{1000} \times \text{dilution factor} \times 100 = \text{mg iodine per 100 cc urine.}$$

11708

Degree of Ketosis During Fasting.

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Ketosis during extended fasting has heretofore been measured by the ketonuria. Because the acetone bodies are threshold substances the ketonuria does not necessarily give a true picture of the degree of ketosis which exists. Furthermore the only record of even the ketonuria in fasts of more than 3 or 4 days' duration were obtained prior to the development of modern methods for determining acetone bodies. We have examined the degree of ketosis during fasting in a human subject and in a group of rats through measurements twice daily of the level of acetone bodies in the blood. In the rats arterial blood and in the human subject venous blood was used for this purpose. Due to the utilization of acetone bodies by the tissues during a ketosis[1] the figures for acetone bodies for venous blood