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A Rapid Micro Method for Determining Diodrast and Inorganic Iodide Iodine in Blood and Urine.*

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The present paper describes a rapid micro method for the determination of diodrast or inorganic iodide iodine in whole blood, blood cells, plasma or urine. Hundreds of determinations of diodrast and inorganic iodide added to water, to urine and to filtrates have shown substantially a 100% recovery. With the method in its present form not all of the iodine is recovered from some other iodine compounds investigated, skiodan, iodeikon and isoiodeikon. The method has not been applied to normal blood or urine. The diodrast and skiodan

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used in this work were kindly furnished by the Winthrop Chemical Company; the iodeiokon and isoiodeikon by the Mallinckrodt Chemical Company.

Principles of Method. The iodine-containing sample is digested in an acid solution of potassium permanganate, the iodine thereby being oxidized to iodate and the organic matter destroyed. The excess permanganate is reduced by addition of nitrite and the excess nitrite then destroyed by urea. The iodate is then titrated against a standard 0.0004715 N thiosulphate solution in the presence of an excess of potassium iodide.

Procedures. With urine the determination is carried out on a diluted sample. With plasma it may be done either on 0.1 cc (or less, depending on iodine content) of plasma or on a sample of plasma filtrate. Because of their higher organic matter content it has not been found practicable to digest unprecipitated whole blood or cells; these determinations must be done on filtrates, where most of the organic matter has been removed. While determinations can be carried out on unprecipitated plasma, we usually use plasma filtrates, particularly at low iodine levels, since with these an amount equivalent to a larger amount of plasma can be used. With urine no precipitation is ever required.

Procedure for determination on filtrates of blood, plasma or cells.

Reagents: 7% and 15% trichloracetic acid.
0.4M potassium permanganate.
4N sulphuric acid.
1.0M sodium nitrite.
5.0M urea.
Crystalline potassium iodide.
1% starch.
0.0004715N sodium thiosulphate.

Precipitation. Take one volume of blood, plasma or cells, 6 volumes of water and 3 volumes of trichloracetic acid (7% for blood or plasma, 15% for cells) in a small Erlenmeyer flask, shake, let stand 5 minutes and filter. On working with blood or cells, the cells are laked by the water before the trichloracetic acid is added.

Digestion. (a) *Individual tubes by hand.* Take 3 cc of filtrate in an 18 x 150 mm test tube, add 6 drops (0.3 cc) of 0.4M permanganate and 2 drops (0.1 cc) of 4N sulphuric acid. With very low iodine levels (less than 1 mg I per 100 cc) 5 cc of filtrate may be used, in which case 0.5 cc of 0.4M permanganate and 3 drops of 4N sulphuric acid are added. With high iodine levels, if less than 3 cc of filtrate is used, the volume should be brought up to about 3 cc by adding distilled water before the digestion; here 2 or 3 drops of 4N

sulphuric acid and 0.3 cc of 0.4M permanganate are used. With constant shaking, heat for 3 to 3.5 minutes over a micro Bunsen burner with flame 3.5 to 4.5 cm high and protected from drafts. Shaking should be a fine wrist motion rather than a slow undulation; keep digest constantly agitated to avoid local overheating. Boiling should begin in about 40 seconds and should then be almost continuous, with tube being continually brought into and out of flame and not in the flame more than half the time; the digest should never be permitted to boil more than one-third the distance up the tube. Avoid heating on side of tube above fluid line. After digestion add 4 or 5 drops (0.25 cc) 1.0M sodium nitrite (to 1 drop excess) drop by drop to hot digest; drop nitrite directly into digest, not running down wall. Digest will clear immediately on shaking. Heat with shaking for 1 minute, shaking so as to wash down above highest level reached by digest during digestion. Next add 4 drops 5.0M urea. Shake and heat for 2 minutes; this includes time required for tipping and rolling tube to wash down above highest level reached by nitrite. During the nitrite and urea treatments the solution should be boiled gently part of the time.

(b) *Digestion of number of tubes simultaneously in water bath.* Put tubes in a rack into a boiling water bath for 10 minutes; it is not necessary to shake during this interval. Level of water in bath must be above level of digest in tubes. Tubes need not be covered; with appropriate size of flame no difficulty should be encountered by water from bath splashing into tubes. If rack is suspended with its bottom slightly above bottom of bath, danger from bumping and splashing is abolished, although this precaution is usually not necessary. Preparation of tubes for digestion is same as with individual digestions. With either hand or water bath digestion the permanganate should not be added until shortly before digestion begins. After 10 minutes lift rack from bath and add nitrite, shaking each tube on addition of nitrite. Return rack to bath for 2 minutes. During this 2 minutes the analyst continuously makes the rounds of the tubes, lifting out a tube in each hand, giving them a shake, returning them to the bath and passing on to the next pair. With 10 tubes one should get around 3 or more times in 2 minutes. After this 2 minutes again remove rack from bath and add urea, with appropriate shaking and washing down, to each tube. Again return rack to bath for 2 minutes, with seriatim shaking as during the nitrite treatment. Finally remove rack from bath, shake tubes once more, and cool.

Titration. Put tubes in a rack into a cold water bath (5-10°C) for a few minutes. After cooling, remove from water and titrate

in an artificially lighted room, using a photoflood bulb or other source of white light. For titration, add a few crystals of potassium iodide and titrate with 0.0004715N thiosulphate, 1 drop of starch solution being added toward end of titration. Each microgram of iodine titrates 0.1 cc. The mean titration error in a triplicate series is not more than 0.003 cc, the maximum not more than 0.005. A blank on normal filtrate should show zero titration. We use a burette graduated to hundredths of a cc and read to thousandths with a hand lens. The burette tip is washed with 2 or 3 drops of water from a medicine dropper after each delivery near the end of a titration.

Reactions. The permanganate oxidizes the organic matter and carries the iodine to iodate. The nitrite destroys the excess permanganate and manganese dioxide, forming the manganous salt of the acid present. At proper acidity and with reasonably judicious treatment, *i. e.,* avoidance of excessively vigorous or lengthy heating, the nitrite does not at all reduce the iodate.

$$2KMnO_4+5HNO_2+3H_2SO_4=K_2SO_4+2MnSO_4+5HNO_3+3H_2O$$

The excess nitrite remaining after permanganate is destroyed would itself liberate iodine from potassium iodide; it must, therefore, be destroyed before the titration. While nitrite reduces permanganate it oxidizes urea, with destruction of both nitrite and urea. The excess urea remaining after destruction of the nitrite does not interfere with the titration, nor does it reduce iodate.

$$CO(NH_2)_2+2HNO_2=CO_2+2N_2+3H_2O$$

Procedure for determination on urine. With urine, dilute so that 3 cc contains from 5 to 15 micrograms iodine. Take 3 cc of dilution in test tube, add 0.3 cc of 0.4M permanganate and 0.15 cc of 4N sulphuric acid and proceed as with filtrate above, except that with water bath digestion only 5 minutes of digestion are required. A normal adult with a surface area of 1.73 sq. M. and with a plasma level of 2 mg diodrast iodine per 100 cc will have a urinary output of 8 to 12 mg iodine per minute.

We have carried out several hundred determinations of known amounts of diodrast and of potassium iodide added to water, to urine and to filtrates. In these filtrate analyses the diodrast or iodide was added to the filtrates; they are distinct from the determinations presented in Tables II and III, where diodrast was added to blood, plasma or cells before precipitation. There is a complete recovery from water and urine and from plasma, blood and cell filtrates of human, dog and horse bloods. A few representative findings are shown in Table I. These results have been obtained with amounts of iodine in the sample analyzed ranging from 2 to 15 micrograms; there may be a slight loss of iodine when more than 15 micrograms

TABLE I.
Recovery of Diodrast and Inorganic Iodide Iodine from Water, Urine, and from Filtrates.

Material	Iodine in sample analyzed, μg	Iodine recovered, μg	% recovery
Aqueous solution of diodrast	6.00	6.00	100.0
	6.00	5.96	99.4
	8.00	8.04	100.5
	12.00	11.90	99.2
	12.00	12.02	100.2
Diodrast in urine	9.27	9.32	100.5
	9.20	9.15	99.5
" " human plasma filtrate	6.00	6.04	100.7
	6.00	6.00	100.0
	12.00	11.93	99.4
" " whole blood filtrate human	6.00	6.08	101.2
horse	8.00	7.91	98.9
Potassium iodide in horse whole blood filtrate	6.00	6.04	100.7

is taken. We can, therefore, say that the above procedure will determine quantitatively the iodine present in the sample analyzed. With urine this means all the iodine present but with blood, plasma or cell filtrates the question arises whether all the diodrast comes through in the filtrate. This point has been investigated at some length on horse, dog and human bloods.

The percentage of diodrast coming through in the filtrate has been determined at various iodine levels for whole blood, for plasma and for cells. The outline of a typical experiment follows: With a given sample of blood, 3 trichloracetic precipitations are made on whole blood, 3 on plasma and 3 on cells, where a known amount of diodrast has been added to the blood, the plasma and the cells, respectively, before precipitation. From 3 to 6 iodine determinations are made on each of the 9 filtrates so obtained. Whole blood and cells are delivered from pipettes to contain and are laked before addition of diodrast. The above procedure is carried out with each of 3 known iodine concentrations. Table II shows the results of such an experiment with a sample of horse blood. Each value in the table represents the average of the 3 to 6 determinations on that filtrate; the maximum deviation from the mean among determinations on a single filtrate is never more than 3%, with a probable error of less than 2% in a triplicate series.

Experiments of the type outlined in Table II have been carried

TABLE II.
Horse Blood 2.
Figures in the Table Designate the Percentage of Diodrast Coming Through in the Filtrates.

		Whole blood	Plasma	Cells
At 2.5 mg I per 100 cc	Filtrate 1	67.0	82.0	49.8
	" 2	64.5	81.2	51.0
	" 3	65.7	82.0	
At 5 mg I per 100 cc	" 1	66.9	82.2	50.6
	" 2	65.2	82.8	51.3
	" 3	66.9	84.3	
At 10 mg I per 100 cc	" 1	64.8	82.5	
	" 2	65.1	81.7	
	" 3	66.1	81.8	
Avg % of diodrast coming through in filtrate		65.8	82.3	50.7

out on 4 horse, 2 dog and 5 human bloods, at iodine levels of 2, 2.5, 4, 5, 8 and 10 mg per 100 cc. It was found that the percentage of diodrast appearing in the filtrates was independent of the iodine concentration within the above range. It was further found that not only is the percentage of diodrast coming through in the filtrate constant with multiple precipitations from a given sample of blood, plasma, or cells but that it is almost constant in all normal horse, dog and human bloods so far examined. The findings are shown in Table III, where a given figure designates the average of the findings on all the filtrates of a given sample of blood, plasma or cells.

The above findings show that with normal human bloods no great error will be introduced by the assumption that the diodrast iodine

TABLE III.
Percentages of Diodrast Coming Through in Whole Blood, Plasma and Cell Filtrates of Horse, Dog and Human Bloods.

	Whole blood	Plasma	Cells
Horse 1	64.3	84.3	
" 2	65.8	82.3	50.7
" 3	63.9	84.5	
" 4			50.8
Dog 1	65.0	85.2	49.4
" 2	69.4	84.2	54.8
Human			
D.N.	66.0	85.0	48.0
Grov.	64.4	84.0	48.3
Loe	64.8	84.0	
W.C.S.	66.9	85.5	54.4
E.W.S.	65.8	84.6	55.3
Avg of humans	65.6	84.6	51.5

content of whole blood is 100/65.6 × that indicated by the filtrate analysis; for plasma the factor is 100/84.6 and for cells 100/51.5. It is of course a simple matter to determine these factors directly for each subject under investigation if the highest accuracy is desired. Since urine is digested without precipitation, all of the iodine in the sample is determined and no factor is used. The calculation here is simple, *i. e.*, each 0.1 cc of titration represents 1 microgram of iodine in the sample analyzed.

Alternative procedure for determination of iodine in plasma. As stated above, the determination may be made on 0.1 cc or less of unprecipitated plasma. In view of the constancy of the percentage of diodrast appearing in plasma filtrates we prefer to use the filtrate procedure, particularly if the level is below 2 mg per 100 cc, since 3 cc or more of filtrate can be used, giving a higher titration. However, the alternative procedure with iodine levels of 2 mg per 100 cc or more can be used, although it is considerably less accurate.

To 1 volume plasma or serum add 19 volumes water. Take 2 cc of this dilution in a 22 x 175 mm test tube, add 1 cc 0.4M $KMnO_4$ and 0.5 cc 4N H_2SO_4. With high diodrast levels correspondingly greater dilutions can be made. Digest 3 to 3.5 minutes by hand. Add 1M nitrite to hot digest drop by drop, shaking with each drop addition. Add 2 drops excess; the total will be 8 to 10 drops. More time will be required in washing down the tube walls than is the case with filtrate, since in the present case there is a greater amount of MnO_2 to be destroyed. The nitrite treatment, including addition of nitrite, shaking, washing down and heating, will take 2 to 3 minutes. Next add 7 drops (0.5 cc) of 5M urea, heat, shake and wash down for another 2 minutes. Titrate as with filtrate. Along with each set of analyses carry out a determination on a known amount of diodrast added to a blank plasma and use the factor so obtained in calculating the unknowns. The departure of this factor from unity will in general be considerably less than with the alkali fusion method of iodine determination. It departs somewhat from unity because, (a) with a less vigorous nitrite treatment a trace of MnO_2 remains undestroyed, raising the titration, (b) with a more vigorous nitrite treatment, sufficient to give a very low blank, small amounts of iodine are likely to be lost (iodate reduced to iodide), lowering the titration. The factor for a given analyst varies within narrow limits; different analysts may show different factors.

With the unprecipitated plasma digestion the error should not be greater than 10%; with continued practice the error can be reduced below this figure but the accuracy attainable with this method will never be as great as with filtrates.