[DIVISION OF PHARMACOLOGY, HYGIENIC LABORATORY, U. S. PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE, WASHINGTON, D. C.]

THE DETERMINATION OF IODINE IN THYROID.

BY ATHERTON SEIDELL.

Received October 11, 1909.

In a recent paper in THIS JOURNAL,[1] by L. W. Riggs, attention is called to a possible source of error in the ordinary Baumann method for the determination of iodine in thyroid. Results are given from which it is concluded that during the fusion of the protein with caustic alkali and sodium nitrate a variable amount of iodate is formed and on this account low results are obtained unless this compound is reduced and its iodine added to that obtained in the usual process.

Although the author's determinations of iodine in samples of thyroid glands show that variable amounts are recovered by the reduction process advocated, it is admitted that in the majority of analyses made of mixtures of potassium iodide and fibrin none or only traces of iodine were recovered by the reduction process. (The analytical results are not given.)

An examination of the results obtained upon thyroids shows that, with only a few exceptions, the larger percentages of iodine recovered by the reduction process are in those analyses in which the smaller amounts of total iodine were involved. It therefore appears that on account of the small amounts of iodine present in these cases the experimental errors were greatest in the particular determinations upon which the author bases his conclusion.

The experience gained by me in this laboratory during the past few years indicated that the Baumann method for the determination of iodine in thyroid is in general entirely satisfactory. Analyses of the same samples of commercial desiccated sheep's thyroid made at various times gave concordant results in every case. Since, however, more positive information than my past experience was considered necessary, a series of determinations was made for the purpose of ascertaining whether the possibility of the formation of iodate could be seriously entertained. The results of these experiments showed that no appreciable loss of iodine could be definitely ascribed to the formation of iodate. The modification of the Baumann method by the introduction of a reduction process as suggested by Riggs is therefore unnecessary and furthermore leads to greater errors in the iodine determination than are inherent in the method as originally suggested by Baumann.

The determinations which are shown in the accompanying table were made by following in general the details described by me in a paper entitled "A New Standard for Use in the Colorimetric Determination

[1] THIS JOURNAL, 31, 710–717 (June, 1909.)

of Iodine."[1] The critical points in the method are fusion to a clear tranquil melt, and care in acidifying the aqueous solution to prevent the loss of iodine during the evolution of the carbon dioxide as the acid is added. Carbon tetrachloride was used in place of chloroform with very satisfactory results. The color of iodine dissolved in the carbon tetrachloride matches that of Fuchsine S dissolved in dilute hydrochloric acid much better than do the chloroform solutions of iodine. The Fuchsine S solutions, however, which have been standardized against iodine dissolved in chloroform can not be used since the same amount of iodine yields a deeper pink in carbon tetrachloride than in chloroform. New Fuchsine S standards were therefore prepared and used in all the determinations. The reduction process which was applied in every case to the acid aqueous solutions after the removal of the carbon tetrachloride layer was carried out exactly as prescribed by Riggs. The determination of the iodine in the solution after the reduction was made by shaking with 2 cc. portions of carbon tetrachloride, filtering 1 cc. of the latter into a small test tube and comparing against 1 cc. standards in similar tubes. These standards were made from Fuchsine S solution to correspond in color to 0.0, 0.005, 0.01 and 0.02 mg. iodine dissolved in 1 cc. carbon tetrachloride.

In the case of the column showing "Mg. I present" the figures for the desiccated thyroids show the results obtained upon these samples two years or more ago. The present determinations are therefore in the nature of checks upon results obtained during my earlier experience with this method.

A glance at the following table shows that in no case was more than 0.03 mg. iodine obtained by the reduction process. In those cases where greater care was exercised in removing all traces of iodine before subjecting the solution to the reduction (*i. e.*, with the use of 4 portions of CCl_4) no iodine at all was obtained. In the duplicate determinations made with all conditions identical except the omission of the Devarda's alloy the amount of iodine was practically the same as found with the use of the alloy for reduction. It therefore appears that the small amounts of iodine found after the reduction are not derived from iodate but are simply the residual amount which the several extractions with carbon tetrachloride have failed to remove. In order to test this point more carefully the amounts of iodine extracted by each successive 3 cc. portion of carbon tetrachloride in the case of determination No. 18 were estimated. They were: First 3 cc. portion contained 0.06 mg. I; second, 0.03 mg.; third, 0.01 mg.; and fourth, none. The subsequent reduction of this solution by means of Devarda's alloy yielded no appreciable amount of iodine.

[1] *J. Biol. Chem.*, **3**, 391–3 (Oct., 1907).

TABLE SHOWING RESULTS OBTAINED BY REDUCING WITH DEVARDA'S ALLOY THE AQUEOUS SOLUTION LEFT FROM THE IODINE DETERMINATION IN THYROIDS.

Det. No.	Sample	Mg. I present.	Mg. I found.	No. of 2-3 cc. portions of CCl_4 to remove residual I from aqueous layer.	Gram Devarda's alloy to reduce alkaline solution.	Mg. I recovered from solution after making strongly acid.
1	10 cc. KI solution	1.0	1.0	2	0.5	0.03
2	10 cc. KI solution	1.0	1.0	3	0.5	0.00
3	10 cc. KI solution	1.0	1.0	3	None	0.00
4	0.5 g. Thyroid No. 106[2]	0.98[1]	0.98	2	0.5	0.015
5	0.5 g. Thyroid No. 106	0.98	0.91	3	0.5	0.03
6	0.5 g. Thyroid No. 106	0.98	0.99	3	None	0.03
7	1.0 g. Thyroid No. 116	1.1[1]	1.05	2	0.5	0.03
8	1.0 g. Thyroid No. 116	1.1	1.02	3	0.5	0.03
8	1.0 g. Thyroid No. 116	1.1	1.02	3	None	0.03
10	0.5 g. Thyroid No. 116 + 0.5 cc. KIO_3 sol	1.0	0.97	3	0.5	0.02
11	0.5 g. Thyroid No. 116 + 0.5 cc. KIO_3 sol	1.0	0.94	3	None	0.015
12	1.0 g. Dried Beef + 1.0 cc. KIO_3 sol	1.0	0.98	3	0.5	0.03
13	1.0 g. Dried Beef + 1.0 cc. KIO_3 sol	1.0	0.92	3	None	0.02
14	0.5 g. Thyroid No. 99	0.90[1]	0.88	4	0.5	0.01
15	0.8 g. Thyroid No. 105	0.88[1]	0.87	4	0.5	0.00
16	0.5 g. Thyroid No. 109	0.65[1]	0.64	4	0.5	0.00
17	0.5 g. Thyroid No. 109*a*	0.55[1]	0.63	4	0.5	0.00
18	0.4 g. Thyroid No. 121	0.40[1]	0.43	4	0.5	0.00
19	0.25 g. Thyroid No. 117	0.27[1]	0.25	4	0.5	0.00

Another point of interest shown by the results in the table is that the iodate itself is completely converted to iodide and yields all of its iodine by the ordinary Baumann procedure. In the four cases in point, determinations No. 10, 11, 12 and 13, a known amount of potassium iodate in the form of a standardized aqueous solution (containing KIO_3 equivalent to 1 mg. I per 1.0 cc.) when mixed with thyroid or with dried beef showed no trace of iodate in the acid aqueous solution of the melt as measured by the yield of iodine after the reduction process.

Some additional determinations confirming this point made by me nearly a year ago are as follows:

[1] From previous determinations.

[2] The samples of thyroid referred to above (No. 106, No. 116, etc.) are commercial preparations of desiccated sheep thyroid obtained at different dates from two American manufacturers.

Det. No.	Grams cracker dust.	Cc. KIO_3 solution (1.0 mg. I per 10 cc.).	Mg. I present.	Mg. I found.
1	3	10	1.0	0.91
2	2	12	1.2	1.2
3	1	8	0.8	0.82
4	1	10	1.0	1.04
5	1	12	1.2	1.23

The observation reported by Riggs probably resulted from his failure to remove completely the residual iodine from the acid aqueous layer before applying his reduction process. There is a distribution of the liberated iodine between the carbon tetrachloride and the acidified aqueous layer and, since at extreme dilution the constancy of distribution coefficients disappears and immiscible solvents extract successively smaller relative amounts of given substances, it follows that the complete removal of the last portions of iodine from the aqueous layer is a matter of considerable difficulty. This fact therefore plays a very important part in the modified method as proposed by Riggs since any dissolved iodine, not removed before the reduction process, leads to a positive error. In the Baumann method, however, the loss of iodine due to its solubility in the aqueous layer plays no appreciable part, since the standards used for estimating the iodine are prepared under conditions essentially identical with those under which the solution of the iodine of the sample is prepared.

[From the Hull Physiological Laboratory, University of Chicago.]

METHODS FOR THE QUANTITATIVE CHEMICAL ANALYSIS OF ANIMAL TISSUES. I. GENERAL PRINCIPLES.

By W. Koch.

Received October 12, 1909.

General Principles.—The study of the chemical transformations of the body as a whole has of recent years received a great impetus by the careful revision of methods of urine analysis by Folin.[1] Such methods permit, however, of only tentative conclusions as to the processes going on in the individual tissues, and the study of the tissues themselves will become sooner or later a necessity. The time appears particularly favorable for a review of what has been done so far and a consideration of just how much can be accomplished with our present knowledge.

A review of the various attempts at the study of tissues by quantitative chemical methods reveals an unusual amount of misdirected effort. Thus on the one hand we have the physiologist, in an attempt to solve his problems making use of chemical methods of doubtful accuracy; on the other hand, the chemist supplying the physiologist with estimations of admirable accuracy as far as chemical technique is concerned, but

[1] *Am. J. Physiol.*, 13, 45 (1905).