STUDIES IN BLOOD IODINE BY THE USE OF A NEW CHEMICAL METHOD*

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I. History

Harrington[1] and McClendon[2] have reviewed the history of the thyroid gland and the relation of iodine to that gland in a very complete manner. The present review, therefore, will be limited to a few statements especially relevant to the necessity for better technical methods in this field of biochemistry.

Iodine was discovered by Courtois[3] in 1812 and publicly announced by Clement[4] in 1813. Immediately following this publication, medical men observed that many of the substances containing large amounts of iodine were those substances, such as kelp, which had been useful in the treatment of diseases of the thyroid gland. This marked the birth of the study of iodine in connection with goiter, and since that time, biochemical investigations concerning the thyroid gland have been centered largely around the element iodine. Coindet[5] in 1820 used iodine as such in the treatment of thyroid disorders and by most authorities is said to have been the first to make therapeutic use of the new drug. However, a quotation† from Prout[6] indicates that iodine was first used in England in 1816.

"It may not be amiss to observe here that the author of the present volume first employed the hydroiodate of potash as a remedy for goiter, in the year 1816, after having previously ascertained by experiments upon himself, that it was not poisonous in small doses as had been represented. Some time before the period stated, this substance had been found in certain marine productions; and it struck the author that burnt sponge (a well-known remedy for goitre) might owe its properties to the presence of Iodine, as this was his motive for making the trial. He lost sight of the case in which

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† These remarks constitute a footnote in Dr. Prout's paper, which interestingly bears the following rather comprehensive title "Chemistry, Meteorology and the Function of Digestion considered with reference to Natural Theology", by William Prout, M.D., F.R.S., Fellow of the Royal College of Physicians, London, William Pickering, 1834.

"Treatise VIII, of the Bridgewater Treatises on the Power, Wisdom and Goodness of God as manifested in the Creation."

the remedy was employed, before any visible alteration was made in the state of disease; but not before some of the most striking effects of the remedy were observed. The above employment of the compounds of Iodine in medicine was at the time made no secret; and so early as 1819, the remedy was adopted in St. Thomas's Hospital, by Dr. Elliotson, at the author's suggestion."

Ever since these early investigations, almost continuous research on this subject has been in progress and it appears that the greatest obstacle in the way of these researches has been the lack of a reliable method for the estimation of small quantities of iodine. One of many striking examples of this is the work of Chatin.[7] As early as 1853 Chatin had what now appears to be excellent evidence to prove that endemic goiter was always associated with a deficiency of iodine in air, water and soil. In one instance he demonstrated that in Saillon, a village on the Rhone, there was no endemic goiter until after a change of water supply which resulted in a considerable decrease in available iodine. The French Academy of Science investigated the matter and refused to accept Chatin's findings because of frequent contradictions. The Academy of Medicine, however, tested his theory by administering iodine, but used such large doses that the frequent occurrence of iodism caused them to discontinue the experiment.

It was largely on account of the independability of his chemical methods that Chatin was unable to convince his contemporaries that endemic goiter was caused by iodine deficiency. It seems probable that Chatin[8] himself was in grave doubt concerning the correctness of his observations. Therefore, because of the technical difficulties involved in the estimation of iodine, it was not until 1918 that Kimball and Marine,[9] using methods not dependent upon the determination of iodine, showed that endemic goiter was probably due to iodine deficiency in the diet.

It was not until 1914 that anything approaching a reliable method for the determination of iodine was introduced.[10] Kendall devised a modification of Hunter's[11] method which gave accurate results in the determination of iodine in material such as the thyroid gland, which contains large quantities of iodine. This method, however, is not applicable to the study of iodine in blood and tissues, unless enormous amounts of material are used. Ten years later, both von Fellenberg[12] and McClendon[13] published methods for the determination of iodine in organic material. Both these methods have led to definite advances in the knowledge of iodine metabolism, yet neither method is definitely satisfactory.

McClendon's method[13] is accurate but suffers from being too cumbersome for ordinary use. Most of the best studies in blood iodine

have been made with modifications of von Fellenberg's method.[12] Some workers, however, consider that the results are unreliable. Others[14] who believe that the principles involved in von Fellenberg's method are sound, admit that there is definite need for an improvement in the procedure.

Therefore it seemed necessary to reinvestigate methods for the estimation of iodine in blood before researches in this field could progress much further.

II. Preliminary Investigation of Methods for the Determination of Iodine

Before attempting to devise an entirely new procedure it was deemed advisable to investigate all existing methods that seemed at all appropriate. This study was commenced in the spring of 1930. The method of von Fellenberg[12] and several of its modifications were carefully studied. (Aitkin,[15,16] Leitch and Henderson,[17] Closs,[18] Veil and Sturm.[19])

There were two reasons why no modification of von Fellenberg's method was satisfactory and it seemed impossible to modify it in a manner to overcome these inherent difficulties. In the first place, in order to destroy organic material, von Fellenberg heats open crucibles containing the iodine to a dull red heat (i. e. at least 550° C. and probably frequently over 600° C.)

We investigated this problem carefully with the results shown in table I. Each crucible contained 100 micrograms* of iodine in the

Table I

Heat Losses

Test no.	*Micrograms of iodine added*	*Substance used*	*Temperature*	*Time in minutes*	*Amount recovered*	*Per cent loss*
1	100	KI	350°	30	100.4	0.4
2	100	KI	350°	30	100.8	0.8
3	100	KI	400°	30	92.7	7.3
4	100	KI	400°	30	97.2	2.8
5	100	KI	400°	30	94.0	6.0
6	100	KI	450°	30	75.6	24.4
7	100	KI	450°	30	80.6	19.4
8	100	KI	450°	30	76.6	23.3

form of potassium iodide and one c.c. of a saturated solution of sodium hydroxide. At 450° C. during one-half hour there was a 20 per cent loss of iodine and the loss was somewhat variable, possibly

* A microgram is 1/1000 of a milligram and is frequently called a gamma.

due to differences in temperature in various parts of the oven. In the same length of time there was a definite loss even at 400° C. The thermocouple registering the oven temperature was in the hottest part of the oven and when the room was darkened none of the crucibles appeared red.

In the second place, the method of von Fellenberg and the modifications mentioned above involve a technic which most laboratory workers find extremely difficult and they usually fail to master it without several months of constant practice.

Although many workers continue to use this method and get valuable results, von Fellenberg himself apparently recognized the necessity for improvement as is manifest by his publication of a new method in 1930.[20]

His new procedure is more satisfactory than the older method but still is not adequate for present requirements. The material is heated to a dull glow for fifteen minutes and hence there is considerable loss of iodine. The technical difficulties of the former method are avoided to a certain extent but the procedure now requires special apparatus with which only one determination can be made at a time. This precludes its use as a routine measure in a blood chemistry laboratory.

Turner[21] described a colorimetric method for the determination of iodine. This method failed to give satisfactory results in the laboratories of the Cleveland Clinic and Turner[22] apparently experienced considerable difficulty with it, according to a later publication. Prof. E. C. Dodds[23] states that similar principles were employed in his laboratories in attempts to estimate iodine, but the methods failed to give satisfactory results.

In order to avoid the losses of iodine which are encountered by von Fellenberg's method, numerous authors have employed a method of closed combustion. The first method of this type that we investigated was that of Karns.[24] The results secured by this method are definitely too high. The inaccuracy is due to incomplete combustion of organic material. The results were so erroneous in our experience that the method had to be discarded. We considered attempting a recombustion of the material absorbed following the first incineration but this made the process so cumbersome that it did not seem desirable to attempt it.

Foster[25] uses Leipert's[26] modification of Pregel's[27] method and obtains satisfactory results. Metzger and Baumann[28] successfully use a closed combustion method. These procedures, particularly the latter, require special apparatus and for this reason were discarded. Moreover, both methods, when applied to blood, involve the slow process of drying. Therefore it seemed desirable to attempt to

create a new and more satisfactory method for the determination of iodine in biological material. The following criteria were specified: *The method must be rapid, the results accurate, the technic not unusual and the apparatus simple.* An additional aim was to devise, if possible, a method by means of which one operator could make several determinations at one time.

The principles involved are relatively simple and have previously been applied in the Kjeldahl procedure for the determination of nitrogen. The main technical difficulty in measuring nitrogen or iodine has been the separation of an element from substances which interfere with its subsequent estimation by orthodox chemical methods. In the Kjeldahl method, nitrogen is estimated as ammonia and since it is non-volatile in acid solution but volatile from an alkaline solution, it is possible to destroy the organic matter by acid digestion and to remove the nitrogen and ammonia from the remaining mixture by alkalinization, followed by distillation. The properties of iodine are just the opposite. It is non-volatile, within a limited temperature range, in alkaline solution, but can be distilled quantitatively from an aqueous acid solution. Hence it is possible to isolate iodine for estimation by alkaline digestion followed by acid distillation.

At first, in an attempt to avoid the process of alkaline digestion, we tried to liberate the iodine from the blood with sulphuric acid and hydrogen peroxide, to distill it off into a suitable absorption train and then to estimate it by one of the common chemical procedures. This attempt failed completely because large amounts of organic material were distilled off with the iodine and interfered with the subsequent steps. It was only after extensive research along these lines that we discovered that Pfeiffer[29] had published a report stating that he had satisfactorily perfected this procedure. Shortly after his first publication, however, Pfeiffer also discovered that the method was not practicable and published two other papers[30 31] in which he advised conducting the gas from the distillation flask through a combustion furnace in order to complete the destruction of organic material. If large amounts of fat are present in the specimen, the method will not give satisfactory results, and in any case it is cumbersome and requires considerable special equipment. These investigations on technic were then continued with the results shown below.

III. A Method for the Determination of Iodine in Biological Material

A. Fusion with Potassium Hydroxide

Ten cubic centimeters of blood are placed in a 250 c.c. nickel crucible together with 10 c.c. of a saturated solution of potassium

hydroxide, and are heated gently over a Bunsen flame. At first there is excessive foaming and either the burner must be manipulated under the crucible or the crucible must be manipulated over the flame. In any case, it is probably wise for the chemist to wear spectacles during this procedure, because careless manipulation may result in spattering, although this contingency is never encountered by experienced, careful workers. When the foaming abates somewhat, the organic material is washed from the sides of the crucible with a small quantity of water. The boiling is continued for a few minutes, and the walls of the crucible are washed two or three times with small quantities of water. Then the mixture is boiled until foaming ceases. This procedure requires about fifteen minutes.

The crucible is placed in a muffle furnace at 250° C. for thirty minutes. During this process, volatile and inflammable gasses are driven off. With experience, one can recognize the appearance of the fused material when this change is complete so that frequently the crucible need remain at 250° C. for not more than fifteen or twenty minutes. If the temperature is increased too soon, inflammation occurs. The temperature is increased to 360° C. and is kept there for ten minutes. Then the crucibles are removed from the oven.

B. Extraction with ethyl alcohol

Sufficient water is added to the crucible to dissolve all the easily soluble material, the lumps being broken down with a stirring rod. The water is boiled off gently, until a fluid paste is formed on cooling and stirring with the stirring rod. Twenty-five c.c. of 95 per cent alcohol are added. On stirring, the paste forms a fluid sludge which separates from the alcohol in the bottom of the crucible. Any material adhering to the sides of the crucible can be scraped down into the sludge with the stirring rod. After some minutes of stirring, the alcohol is decanted off into a 250 c.c. nickel crucible containing 1 c.c. of a saturated solution of potassium hydroxide. The sludge is then leached four more times with 10 c.c. portions of alcohol. If, during this procedure the sludge forms a thick paste, it is moistened with a few drops of water. After extracting, the sludge contains none of the iodine. The alcohol is evaporated on a steam bath and the contents of the crucible are dried gently over a free flame.

C. Ashing

The crucible is placed in a muffle furnace at 385° C. for ten minutes. A stream of oxygen (2 liters per minute) is passed through the oven during the process of ashing.

D. Purification by Distillation

The residue in the crucible is dissolved in water and filtered into a 500 c.c. Claissen flask. The distillation apparatus is arranged as indicated in Figure 1. The end of the tube leading from the con-

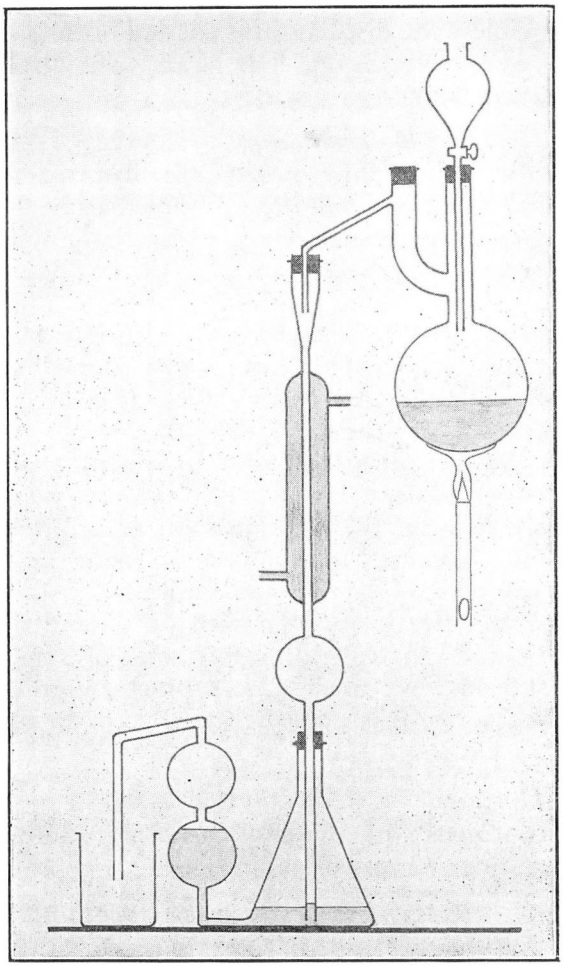


Fig. 1

denser dips under the surface of a mixture consisting of 25 c.c. of water, 0.5 c.c. of a 10 per cent solution of sodium bisulphite contained in the 250 c.c. Fresenius absorption flask. Five c.c. of a 50 per cent solution of sulphuric acid, 2 c.c. of a 10 per cent solution of ferric sulphate, and 2 c.c. of a 30 per cent solution of hydrogen peroxide are added through the dropping funnel. More acid is added if necessary to make the solution strongly acid. The presence

or absence of ferric hydroxide acts as an indicator. The total volume of the solution at this time should be about 250 c.c. This is distilled for one-half hour with frequent additions of a 30 per cent solution of hydrogen peroxide. At the end of this step, the volume of the solution in the Claissen flask should have been reduced to about 50 c.c. Unless the distillation is carried on briskly not all the iodine will be carried over in one-half hour. The distillate is transferred to a 600 c.c. beaker.

E. Determination of Iodine

Carbon dioxide and sulphur dioxide are liberated from the distillate by boiling for three minutes. Immediately a 10 per cent solution of potassium hydroxide is added until the solution is alkaline to litmus paper. Not more than 1 c.c. of the potassium hydroxide should be required. The alkaline solution is then boiled and evaporated to a volume of about 10 c.c. and this is transferred to a 50 c.c. Ehrlenmeyer flask by washing the beaker three times with small quantities of iodine-free water. One drop of methyl orange is added and the solution is neutralized with a 3 per cent solution of sulphuric acid. Three drops of sulphuric acid in excess of the amount required for neutralization are added, and then one drop of bromine. Upon shaking, the solution should turn yellow immediately. Then it is evaporated to a volume of about 2 c.c. One drop of a one per cent solution of starch and a minute crystal of potassium iodide are added and titration is done with a thousandth normal solution of sodium thiosulphate. A microburette or a pipette may be used for the titration. We use a 0.2 c.c. pipette graduated in thousandths. With practice in a good light the error in titration is not more than 0.002 c.c.

F. Calculation

One cubic centimeter of thousandth-normal thiosulphate solution equals 21.2 micrograms of iodine.

G. Reagents and Their Purification

1. *Distilled Water.* Distilled water is made distinctly alkaline with potassium hydroxide and redistilled from a glass still.

2. *Potassium Hydroxide.* A saturated solution of potassium hydroxide is used, which can be purified by washing in a separatory funnel with iodine-free alcohol. It is essential that the solution of potassium hydroxide be saturated during this procedure.

3. *Iodine-free Alcohol.* Iodine-free alcohol is prepared by distilling an alkaline solution.

4. *Sulphuric acid, 50 per cent.* Iodine may be removed from sulphuric acid by heating the concentrated solution to 150° to 200°

C. for five hours. This then is diluted 50 per cent with iodine-free water.

5. *Ferric Sulphate.* A 10 per cent solution of ferric sulphate in 1 per cent sulphuric acid is purified by boiling.

6. *Hydrogen Peroxide, 30 per cent.* Hydrogen peroxide is purified by boiling for a few minutes under reduced pressure. We have never found iodine in peroxide.

7. *Potassium hydroxide, 10 per cent solution.*

8. *Methyl orange, 1 per cent solution.*

9. *Sulphuric acid, 3 per cent solution.*

10. *Sodium Bisulphite, 10 per cent solution.* The bisulphite solution usually does not contain a sufficient amount of iodine to interfere with the method, but if necessary, it can be purified by washing the dry powder with alcohol.

11. *Bromine.* Many laboratory workers have had difficulty in the purification of this reagent, and others have ignored the large quantities of iodine which it usually contains. Iodine can be removed from bromine by the following simple procedure: 50 c.c. of bromine are placed in a retort and covered with a layer of 10 per cent copper sulphate, about 1 cm. deep. The bromine is distilled through the sulphate solution into an ice-cooled container.

12. *Sodium Thiosuphate, thousandth-normal—N/1000.* This solution should be kept away from light.

13. *Starch, 1 per cent solution.*

14. *Potassium iodide.*

H. Discussion

The method as outlined above requires about three hours from the time the blood is taken until the results are obtained. Thus the technic is much more rapid than most of the available procedures. Several determinations can be made simultaneously by one technician. The method can be greatly simplified when used for the determination of substances containing larger amounts of iodine and less organic material.

The thiosulphate should be standardized against potassium iodate, using quantities of iodine similar to those to be estimated. The standardization should be checked every two or three days. All titrations should be done under the same conditions of light, fluid volume, temperature and acidity. Since bromine oxidizes potassium iodide with the liberation of iodine, it is wise, if possible, to carry the flask to a different room immediately after boiling off the bromine, in order to complete the procedure away from bromine fumes.

It is possible frequently to omit the alcohol extraction, the object of which is to avoid the necessity of prolonged ashing which results

in the loss of iodine. During the fusion all of the iodine in organic combination is converted to the inorganic form. If organic material is present in large quantities, as in blood, it interferes with the subsequent steps. Only a small proportion of this organic material is carried over with the alcohol.

On very rare occasions, even when the above technic is carefully followed, enough organic matter may be carried into the distillation flask to interfere seriously with the liberation of iodine. The presence of such an amount of organic material can be recognized by an unusually dark color of the solution resulting from reduction of the ferric sulphate. This difficulty can be successfully overcome by the addition of an excessive amount of a saturated solution of potassium permanganate to the hot mixture before distilling and before hydrogen peroxide is added, as this undergoes double decomposition with the permanganate.

If only a comparatively small amount of organic material is present, as in the case of diiodotyrosine or even thyroid tissue, it is possible to omit the alcoholic extraction completely.

A blank should be run on all reagents frequently, and the blank value must be subtracted each time a determination is done. Frequently this value is 0.0 and should never be more than 0.2 micrograms.

Table II presents a series of results showing the determination of

TABLE II

Estimation of Iodine (Whole Procedure)

Test no.	*Substance used*	*Micrograms of iodine added*	*Amount of iodine recovered*	*Per cent of recovery*
1	KI	1.05	1.05	100
2	KI	1.05	1.05	100
3	KI	1.05	1.01	96
4	KI	1.05	1.01	96
5	KI	2.1	2.12	101
6	KI	2.1	2.14	102
7	KI	2.1	2.02	96
8	KI	2.1	2.01	96
9	KI	2.1	1.89	90
10	KI	2.1	2.08	99
11	KI	5.25	4.91	93
12	KI	5.25	5.02	95
13	KI	5.25	5.40	103
14	KI	5.25	5.06	96
15	KI	5.25	5.02	95
16	KIO_3	2.1	2.03	97
17	KIO_3	2.1	2.10	100
18	KIO_3	2.1	2.14	102
19	KIO_3	2.1	2.14	102
20	KIO_3	2.1	2.14	101
21	KIO_3	2.1	2.16	103
22	KIO_3	2.1	2.12	101
			Average recovery	98.4%

iodine when known quantities of potassium iodide and potassium iodate were used. The average recovery of 98.4 per cent and the regularity of results is gratifying.

Table III shows a series of results obtained by using known

TABLE III

Estimation of Iodine (Whole Procedure)

Test no.	*Substance used*	*Micrograms of iodine added*	*Amount of iodine recovered*	*Per cent of recovery*
1	Para-iodo-benzoic acid	.79	.77	98
2	"	.79	.73	92
3	"	.79	.79	100
4	"	3.95	3.95	100
5	"	3.95	3.84	97
6	"	3.95	3.82	97
			Average recovery	97%
7	Di-iodo-tyrosine	4.60	4.52	98
8	"	4.60	4.60	100
9	"	4.60	4.56	99
			Average recovery	99%

amounts of diiodotyrosine and paraiodo benzoic acid, which are quite comparable to those obtained by the use of inorganic compounds.

The most commonly accepted single criterion for the accuracy of a microchemical method as applied to biological material is the recovery of added quantities of the chemical in question from blood, tissue, or food. Success in this regard, as shown in Table IV, increases confidence in the procedure.

TABLE IV

Recovery of Iodine Added to Blood

Test no.	*Micrograms of iodine in blood*	*Micrograms of iodine added*	*Micrograms of iodine found*	*Micrograms added iodine recovered*	*Per cent added iodine recovered*
1	0.79	2.1	2.81	2.02	97
2	0.79	2.1	2.71	1.92	91
3	0.79	2.1	2.71	1.92	91
4	0.79	2.1	2.85	2.06	98
5	0.79	2.1	2.81	2.02	97
6	0.71	5.25	5.84	5.13	98
7	0.71	5.25	5.75	5.04	96
8	0.71	5.25	5.48	4.77	91
				Average recovery	95%

The question of loss of iodine by evaporation has been investigated. The amount of volatilization is a function of both time and temperature. The loss in ten minutes at 385° C., in the presence of excessive quantities of potassium hydroxide, from material containing such minute quantities of iodine, is so small that its detection by chemical methods is very difficult. The loss probably never is more than 2 per cent.

Hydrogen peroxide is a particularly suitable reagent to use during the distillation procedure, since it liberates iodine from all its combinations. Iodides are quantitatively oxidized by hydrogen peroxide with the formation of free iodine. Iodates and periodates undergo double decomposition with the liberation of free iodine, and in addition the iodine in organic combination is liberated by hydrogen peroxide in strongly acid solution.

The Winkler method of titration which is used in this and several other methods is valuable but is likely to be treacherous. In this procedure iodine is oxidized to iodate which in turn is used to liberate iodine from iodide in acid solution. As a result six times the original amount of iodine is available for titration. The reaction, however, is not specific for iodine. Any substance which will liberate iodine from iodide after oxidation will yield positive results. Many organic compounds have this property. It is for this reason that some of the closed combustion methods such as that of Karns[24] give high results. Ferric iron will also liberate iodine from iodides and will cause erroneously high values. It is our opinion that all the modifications of von Fellenberg's[12] original procedure are likely to err in this regard. Distillation in glass as indicated in the method described in this report should be of great value in precluding such possibilities.

IV. Some Studies Now in Progress in Which the New Method Has Been Employed

A. The Diagnosis of Hyperthyroidism

As the name indicates, the clinical syndrome known as hyperthyroidism is characterized chiefly by symptoms which are referable to hyperactivity of the thyroid gland. Since it is the function of this gland to produce a hormone which contains iodine, it is to be expected that the iodine content of the blood should increase together with the increase in the rate of glandular activity. That this is actually the case was observed several years ago by European workers (Holst and Lunde,[32] and Veil and Sturm[19]) and more recently in this country by Turner[33] and Curtis, Davis and Phillips.[34]

There is some question as to whether or not the level of blood iodine is always elevated in hyperthyroidism. Curtis[34] has stated that, "In patients suffering with diffuse hyperplastic goiter, presenting the characteristic symptoms of hyperthyroidism and with varying degrees of exophthalmos, the blood iodine is consistently elevated." Turner expressed the belief, after examining fifteen patients suffering from hyperthyroidism, that only 66 per cent show an increase in blood iodine. The level of blood iodine in relation to hyperthyroidism has been reinvestigated.

By the examination of twenty normal persons during the winter months, the level of iodine in the blood was found to vary between 6 and 9 micrograms per 100 c.c. of whole blood. This compares favorably with the normal figure of 8.3 micrograms per 100 c.c. established by Veil.[35] It is generally agreed that the normal level is higher in the summer. This is in accord with our findings, although we have not yet sufficient data to establish the normal figure for summer in Cleveland. Veil's summer figure for Munich is 12.8 micrograms per 100 c.c. It is not improbable that there is some geographic difference to be found in the blood iodine level. Cleveland is in a district in which the average iodine intake is definitely low. The normal blood iodine level here may therefore be somewhat lower than in districts where iodine is plentiful. In women there is a sharp increase in the level of blood iodine during the first few days of a menstrual period. During the mid-menstrual period the normal level of blood iodine is the same in women as in men. These factors must all be taken into consideration in interpreting the results of blood iodine estimation.

Table V shows the levels of blood iodine and basal metabolic rates of patients in whom clinically typical hyperthyroidism was present.

TABLE V

Comparison of Blood Iodine and Basal Metabolic Rate in Patients with Hyperthyroidism

Case no.	*Micrograms per 100 c.c. blood iodine*	*Basal metabolic rate*
1	19.5	+37
2	24.2	+23
3	17.9	+ 4
4	24.2	+34
5	38.7	+53
6	49.8	No basal rate
7	17.9	+14
8	21.5	+57
9	11.1	+70
10	23.7	+19
11	20.9	+70
12	42.4	+58
13	25.6	+34
14	28.4	+60
15	38.9	+51
16	15.3	+22
17	21.2	+49

Most of these patients were operated upon and hyperplastic glands were found to be present. In every case there was a definite increase in blood iodine. The level of blood iodine does not appear to parallel closely the severity of symptoms. There is, however, no question

regarding its definite diagnostic value. It will be noted that in Case 9 the level of the blood iodine (11.1 micrograms per 100 c.c.) approached that of the normal which is 6 to 9 micrograms per 100 c.c. This patient had been suffering from severe attacks of hyperthyroidism for several months. In the condition of hyperthyroidism there is not only an increased level of blood iodine, but also an abnormally high excretion of this element. The excretion may be so large that the patient has a negative iodine balance. If this continues over a long period of time the individual will, obviously, become depleted of iodine. This is perhaps the explanation of the extraordinarily low level of blood iodine found in the patient mentioned.

These results are obviously in agreement with those of Curtis.[34] Our normal figure is slightly lower than his, but we agree with him in his statement that blood iodine is increased in hyperthyroidism. Turner's[33] statement that 33 per cent of patients suffering from hyperthyroidism show no increase in blood iodine may result from the fact that he employs different diagnostic criteria. It is possible, however, that his iodine determinations are in error in some cases. In this regard it is interesting to note that at one stage in his procedure the directions read as follows: "Ignite the alcohol and allow to burn until the crucible is dry. Heat the crucible over a low Bunsen flame with a to-and-fro motion, the crucible being allowed to glow to a bright red for a few seconds." In our hands both those steps resulted in a definite loss of iodine.

There is little value in a laboratory method of diagnosis if it is useful only in cases in which the clinician is capable of making a diagnosis at a glance. Nothing could be easier than the diagnosis of typical marked exophthalmic goiter. On many occasions, however, the differential diagnosis of hyperthyroidism may perplex even the most experienced and careful observer. The following four cases are exemplary of the type of diagnostic difficulty which may arise.

Case 1. A young woman, aged twenty-four years, complained of weakness, sweating, a persistent tachycardia and a loss of 25 pounds during the year and a half previous to her examination. The patient's basal metabolic rate had been plus 42 per cent before admission to the Clinic, and a diagnosis of hyperthyroidism had been made. We found that the blood iodine was 6.4 micrograms per 100 c.c., a low normal figure. The patient was placed in the hospital and on repeating the basal metabolic rate it was found to be only plus 7 per cent at one time and plus 3 per cent at another.

Questioning elicited the fact that the patient had been under considerable nervous strain during a long period of time and that the

loss of weight could readily be accounted for by loss of appetite and resultant undernourishment. Her hands and feet were cold. It was the consensus of opinion of her physicians that this patient did not have hyperthyroidism but was suffering from nervous exhaustion and tachycardia of functional origin and nervous exhaustion. Infected tonsils contributed to the general condition of exhaustion.

Since no operation was performed, no pathological proof is at hand, but the final clinical diagnosis was in agreement with the results of the blood iodine determination.

Case 2. A man, forty-two years of age, complained of nervousness but of no loss of appetite. Hyperhidrosis was present. On examination, the pulse rate was 90; blood pressure, 200 systolic and 140 diastolic; basal metabolic rate, plus 26 per cent. The level of blood iodine was low, 5.8 micrograms per 100 c.c. For this reason, it was thought that the patient was not suffering from hyperthyroidism. In the hospital the basal metabolic rate decreased to normal within a period of two days without the use of Lugol's solution. It was agreed that the patient's symptoms could be the result of the cardiovascular condition and a diagnosis of essential hypertension was made. There was no operation.

Cases 3 and 4 are in marked contrast to the picture seen in Cases 1 and 2.

Case 3. A married woman, aged forty-two years, complained of enlargement of the thyroid gland, nervousness, and a throbbing heart. She had suffered from hyperthyroidism for seven years and a partial thyroidectomy had been performed at that time. The diagnosis was made difficult by the fact that the patient was gaining weight and that her basal metabolic rate was minus 12 per cent. The blood iodine was 28.3 per cent. This was an almost definite diagnostic sign of hyperthyroidism. Operation was performed and a diffuse adenomatous goiter was removed involving both lobes of the thyroid gland.

Case 4. A woman, thirty-three years of age, complained of nervousness and loss in weight, although she had a good appetite. Physical examination revealed that the thyroid gland was enlarged to two or three times its normal size. Exophthalmos was not present and there were no cardiovascular symptoms suggestive of hyperthyroidism. The patient's hands and feet were cold and the basal metabolic rate was minus 3 per cent. The blood iodine was 18.1 micrograms per 100 c.c. A diagnosis of hyperthyroidism was made. At operation, an adenoma of the right lobe of the thyroid gland was removed, which, microscopically, exhibited definite hypertrophy and hyperplasia.

In concluding the discussion of the diagnostic value of blood iodine in studying thyroid hyperactivity Curtis[34] might again be quoted. He states correctly that "the significance of the blood iodine in thyroid disease is similar to that of the blood sugar in diabetes mellitus, and to that of the blood calcium in parathyroid disease." In the case of blood iodine the significance seems even greater than that suggested by Curtis. Here, for the first time, we have an accurate chemical method for measuring the amount of a hormone in the blood stream and thus can obtain a direct measure of the rate of glandular function.

B. The Diagnosis of Hypothyroidism

A sufficiently extensive study has not yet been conducted to warrant a definite conclusion concerning the diagnostic value of blood iodine studies on patients suffering from hypothyroidism. The changes certainly are not of the magnitude observed in hyperthyroidism. A considerable portion of the iodine found in normal blood is apparently in various stages of anabolism or catabolism and is not physiologically active. This portion remains moderately constant, and varies more with iodine intake than it does with thyroid activity. A limited number of results indicate that, even in patients in whom severe myxedema is present, the total blood iodine may reach the lower normal levels. Curtis, Davis and Phillips[34] state that in clinical hypothyroidism the blood iodine is decreased about 20 per cent below the normal level. In cases of complete extirpation of the thyroid gland the decrease is somewhat greater. Turner[33] found no decrease in the level of blood iodine in a number of patients suffering from hypothyroidism. It is probable that clinical signs and symptoms and the basal metabolic rate will continue to be of greater value in the diagnosis of hypothyroidism than the determination of the total iodine content of the blood. Possibly further investigation will demonstrate that certain fractions of the blood iodine contain all the thyroid hormone. These fractional analyses may solve the problem of the diagnosis of hypothyroidism.

C. A Study of the Iodine Content of the Blood and Spinal Fluid

Table VI portrays the results of the analysis of the blood and spinal fluid of patients who were undergoing encephalographic studies and except in Case 8 no demonstrable disorders of the thyroid gland were present. These findings show that less than 30 per cent of the blood iodine is diffusable. In this regard it is interesting to note that nearly 70 per cent of the blood iodine is in organic combination.

TABLE VI
Comparative Estimations of Blood Iodine and Iodine in the Spinal Fluid

Case no.	*Micrograms of iodine per 100 c.c. of blood*	*Micrograms of iodine per 100 c.c. of spinal fluid*
1	7.5	1.3
2	8.6	3.4
3	8.3	1.7
4	5.9	3.0
5	8.3	1.2
6	7.4	1.2
7	5.5	1.3
8	11.1	4.5
9	6.3	0.8
10	8.4	2.7
11	8.4	1.0
Average	7.7	2.0

Veil and Sturm[19] and Holst and Lunde[32] have demonstrated that, when the thyroid gland becomes pathologically hyperactive, it is largely the organic iodine of blood which increases. Holst and Lunde[32] express the opinion that the iodine which is in combination with protein and hence is insoluble in ethyl alcohol contains the thyroid hormone. The cerebrospinal fluid is usually considered to be a true diffusate from blood. There is evidence to the contrary, but in any case the chemical examination of cerebrospinal fluid probably gives a more truthful picture of the diffusible elements of blood present under physiological conditions than does the examination of an artificial diffusate prepared under conditions which disturb all natural physical and chemical equilibria.

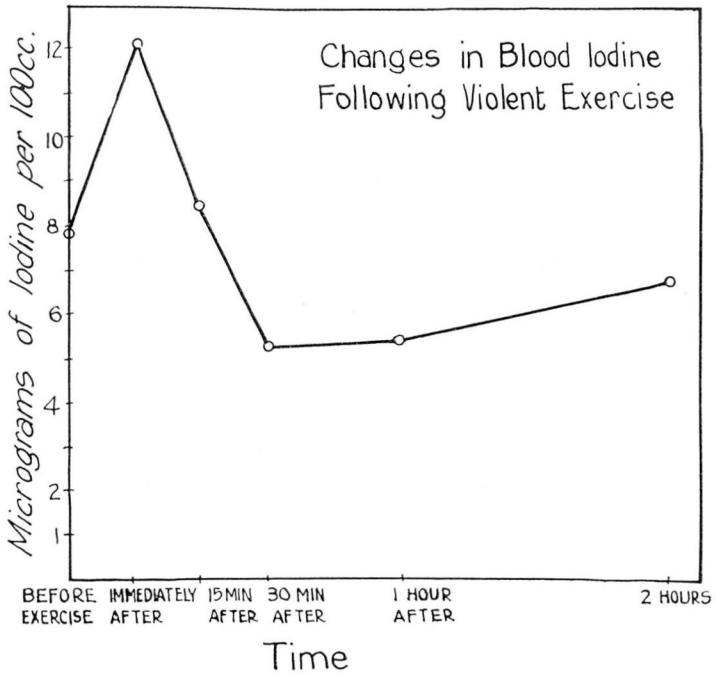
Since the spinal fluid contains but little iodine and the quantity is roughly the same as that of inorganic iodine in the blood it seems probable that no thyroid hormone is present in the spinal fluid.

It is interesting to note that Case 8 in this series was the only one in which there was a high level of blood iodine and the level of iodine in the spinal fluid is definitely the highest in the series. In this patient clinical symptoms of hyperthyroidism were present.

These results are not in agreement with those published by Hahn and Schürmeyer[36], who found an average value of 10.6 micrograms per 100 c.c. of iodine in the blood and 7.4 micrograms per 100 c.c. in the cerebrospinal fluid. According to these results, 69.8 per cent of the blood iodine is diffusible. It may be that in Cologne, on account of the greater amount of iodine in the diet, there is more total and more diffusible inorganic iodine in the blood than in Cleveland. This might explain the discrepancy. The higher values might also be a result of the use of the Winkler titration which, as pointed out above, is likely to give high results in the presence of impurities.

D. The Effect of Exercise on Blood Iodine

The best known function of the thyroid gland is the control of the metabolic rate. For this reason changes in the blood iodine were studied when the metabolic rate was changed in persons with normal thyroid function. Severe acute infections have been shown to cause a rise in blood iodine, but in that case, although the metabolism is increased, the picture is complicated by the presence of bacterial toxins which probably act as thyroid stimulants. It was therefore decided to test the effect, in normal persons, of violent exercise on the level of blood iodine.



The subjects of the experiments were young men between the ages of 20 and 35 years. The exercise lasted for only ten minutes but was vigorous and consisted of running seven times from the ground floor to the eighth floor of a building. At the end of this period the metabolic rate was very high, in one case it was measured and found to be 353 per cent above the normal basal level.

Preliminary experiments showed that there was an immediate and sharp increase in the blood iodine. The changes are being studied in greater detail as shown in Figure 2 and Table VII. The changes in blood pressure, pulse and respiratory rate are indicative of the severity of the exertion.

TABLE VII

The Effect of Violent Exercise on the Amount of Iodine in the Blood

Time	*Blood Pressure*	*Pulse*	*Respiration*	*Micrograms of iodine per 100 c.c. of blood*
Before	110/80	64	21	8.0
Immediately after	135/85	148	40	9.2
15 minutes after	115/82	104	23	5.3
45 minutes after	95/75	88	20	6.3
2 hours after	110/90	76	21	6.8

From these findings it is apparent that alterations in the metabolic rate occasioned by muscular activity do not bring the level of blood iodine to anything like the extreme figures observed in hyperthyroidism. This is of considerable significance in the use of the method as a diagnostic procedure, because it is frequently much more inconvenient to obtain a basal metabolic study than a sample of blood. In this regard we have also observed that blood iodine values are the same before and after eating unless the food contains large quantities of iodine. The prevalence of the use of iodized table salt makes it desirable, if possible, to obtain the blood for the iodine studies when the patient is in a fasting condition.

There is no doubt a marked increase in blood flow during periods of violent activity. This may result in a definite "flushing out" of iodine from the gland. If the entire rise in blood iodine is due to increase in blood flow through the gland one must conclude that in these experiments exercise did not act as a thyroid stimulant. During the period of fatigue which followed the exercise the iodine dropped to subnormal levels. This argues in favor of the idea that exercise and fatigue do, to some extent, alter the rate of thyroid activity.

If this is a real thyroid stimulation it is a matter of interest to see the rapidity with which the gland responds. The first sample of blood was taken immediately on the conclusion of the period of exercise and the iodine level was already elevated. The results show conclusively that the metabolic processes which occur in addition to the basal activity are not quantitatively dependent on the thyroid gland.

E. The Effect of Trauma on Blood Iodine

Trauma in the form of orthodox surgical procedures acts as an astonishingly powerful thyroid stimulant. Major surgical manipulations are followed by a transient hyperactivity of the thyroid gland which develops within a few minutes and continues for a period of less than two to five days in uncomplicated cases. (Table IX.) The amount of iodine which suddenly appears in the blood

TABLE IX

Repeated Postoperative Studies on the Amount of Iodine in the Blood

Case no.	*Preoperative Micrograms of iodine per 100 c.c blood*	*Postoperative Micrograms of iodine per 100 c.c. blood*	*Type of Operation*
1	9.1	45.6	Left adrenal denervation
		43.4	24 hours after denervation
		14.6	48 hours after denervation
		11.3	72 hours after denervation
2	14.17	42.4	Amputation of right breast
		143.1	Amputation of left breast
		6.7	48 hours after operation
		14.1	5 days after operation
3	9.2	95.2	Exploratory laparotomy
		110.0	48 hours after operation
		11.0	5 days after operation

(and therefore no doubt also in other tissues) is of such quantity that the source must surely be the thyroid gland. In none of these cases was iodine used in the operating room.

The extent of the reaction is no doubt somewhat dependent on the susceptibility of the patient to this type of stimulation. The experiments reported in Tables VII and IX do not reveal how much this factor influenced the results. It is, however, apparent from Table VIII that the increase in the level of blood iodine is a function of

TABLE VIII

Changes in Blood Iodine Following Surgical Procedures

Case no.	*Preoperative Micrograms of iodine per 100 c.c blood*	*Postoperative Micrograms of iodine per 100 c.c. blood*	*Type of Operation*
1	9.2	23.8	Catheterization of ureters
2	6.8	23.3	Prostatectomy
3	8.9	36.4	Appendectomy
4	12.1	52.4	Left nephrectomy. D. & C.
5	7.1	61.2	Exploratory laboratory. Cecostomy
6	6.7	8.0	Encephalogram
7	15.3	43.5	Laminectomy
8	8.4	13.7	Encephalogram
9	8.3	20.5	Right adrenal denervation
10	11.9	21.6	Left adrenal denervation
11	14.3	46.3	Left adrenal denervation
12	8.4	24.2	Thyroidectomy. Excision of cyst of abdomen
13	8.3	11.1	Alcohol injection, nerve
14	6.5	6.1	Removal of cataract
15	5.9	19.5	Transurethral resection
16	22.9	45.8	Thyroidectomy
17	6.7	7.1	Hemorrhoidectomy
18	11.8	72.5	Thyroidectomy
19	8.2	10.1	Removal of cataract

the severity of the surgical procedure. In cases 6 and 8 in Table VIII the increase in the quantity of iodine was not small; the operation was minor, viz., encephalography. In cases of removal of a cataract the results were similar. An alcohol injection resulted in an inconsequential thyroid stimulation. Major surgical procedures cause increases of several hundred per cent in the amount of iodine in the blood.

Clinical signs and symptoms have failed to make it apparent to most observers that the thyroid is other than a very sluggish gland. It has not been demonstrated previously that with adequate stimulation the thyroid may react almost as quickly as the central nervous system. The results shown in Table VIII demonstrate clearly that within an hour and a half after any extensive surgical trauma the blood iodine increases to levels observed only in extreme conditions of hyperthyroidism. The speed of this physiological reaction should impress the surgeon again with the necessity for careful preoperative preparation and every postoperative precaution in order to avoid that misfortune known as a "thyroid crisis." The physiological background of the "thyroid crisis" in cases of hyperthyroidism is no doubt associated with the additional strain on the gland imposed by the trauma incident to operation.

Summary

1. The technical difficulties in the study of the biochemistry of iodine are reviewed.

2. A new chemical method for the determination of iodine in biological material is presented, together with evidence for its reliability.

3. The application of chemical methods to the diagnosis of hyperthyroidism is discussed. Patients suffering from the disease exhibit an increase in the level of blood iodine. The value of this laboratory method is emphasized as an aid to differential diagnosis.

4. Spinal fluid contains about one-fourth as much iodine as blood.

5. Exercise causes small increases in the level of blood iodine which falls below normal during fatigue.

6. Surgical procedures cause marked transient thyroid stimulation roughly proportional to the extent of surgical manipulation.

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