THE FATE OF IODIN, IODIDS AND IODATES IN THE BODY

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I. INTRODUCTION

Purpose of the investigation. It is well known that most of the compounds of iodin are excreted in the urine mainly as iodid; and that they circulate in the blood and tissues mainly in this form. Since the systemic effects of all iodin compounds are essentially similar; since they all, for instance, are capable of producing iodism, it is natural to suppose that the effects are produced by the iodid ion. This view, however, is not universally accepted. It has been held by some that iodids may liberate elementary iodin, at least in certain situations and under special conditions. The affinity of the free iodin for proteins, phenols, fats, etc., would then furnish a great variety of reactions. I have not, however, encountered any direct evidence of the liberation of elementary iodin in the tissues; nor any detailed or convincing analysis of the conditions under which this could occur.

Again, there is a rather prevalent opinion that the effects of the various iodin compounds differ at least quantitatively; especially, that there are important differences in the therapeutic efficiency, and in the tendency to produce the phenomena of iodism. Such claims of high therapeutic value with relative freedom from side-actions, are often made by interested manufacturers of special products, on the sole "evidence" of the most superficial type of clinical impressionism. I am not acquainted with any instance in which such a difference has been established

by adequate observation or experimentation. On the other hand, there is no conclusive evidence against these assertions.

It is possible to imagine several ways in which the effects of iodin compounds would differ in detail, even though they eventually circulate as iodid. Such possibilities, however, should not be accepted until they have been investigated. One of these possibilities, the different distribution of the compounds before their conversion into iodid, was studied by McLean (1). Another possibility is the liberation of elementary iodin, previously mentioned. This could be imagined especially for the administration of ele nentary iodin. For instance, it is conceivable that the alkalinity of the body would change the iodin into iodid and iodate or hypoiodite. If such a mixture were actually formed, and if it encountered free acid anywhere in the body, free iodin would be regenerated, which would then attach itself directly to the proteins, fats, etc.

Iodin could also be conceived as being liberated in acid reaction, from iodids in the presence of nitrites; and from iodates in the presence of sulphocyanids.

II. GENERAL PLAN OF INVESTIGATION

The following investigations had for their immediate object to determine whether the above reactions could occur under the conditions of the body; specifically, to answer the questions, (1) whether the administration of elementary iodin can result in the appearance of iodid and iodate in the blood or tissues; and (2) whether elementary iodin can be liberated from iodid and iodate, combined or separately, with the reaction (hydrogen-ion concentration) that is known to exist in the body.

It may be stated at once that both questions are to be answered in the negative.

III. TESTS DEPENDING ON THE LIBERATION OF IODIN CANNOT BE APPLIED DIRECTLY TO SERUM OR URINE

Boehm and Berg, 1876 (2), credit Magendie, 1852, with the discovery that serum and all protein solutions, and also urine,

interfere with the tests for free iodin, unless this is in large excess. The interference is due to the binding of the iodin by the aromatic groups of the proteins, especially tyrosin; and by the phenols and some other constituents of urine. This interference seems to be less well known than it deserves. It is illustrated by the following experiments:

a. Delicacy of the iodid tests. The presence of iodid was tested by the addition of sodium nitrite or potassium iodate, in acid solution, judging the liberation of iodin by the yellow color of the solution, or by the pink color acquired by chloroform, or by the blue color of starch.

The liberation of iodin occurs by the following reactions:

$$(1)\quad HI + HNO_2 = I + NO + H_2O$$
$$(2)\quad 5HI + HIO_3 = 6I + 3H_2O.$$

When the test was performed by mixing 10 cc. of the iodid solution with a few drops of 1 per cent $NaNO_2$ and of H_2SO_4, and shaking with 1 cc. of chloroform, the latter just showed a definite pink if the iodid solution contained NaI 1:150,000.

b. Delicacy of the iodate test. This involves reaction (2). When performed by mixing 10 cc. of KIO_3 solution with a few drops of 1 per cent NaI and of 10 per cent H_2SO_4, and shaking with 1 cc. of chloroform, the limit of sensitiveness was also about 1:150,000.

c. Sensitiveness of iodid-nitrous acid test in protein fluids. Whereas with water, the test (using 10 cc. of the solutions) is positive with NaI 1:150,000, dog's serum requires more than 1:5,000; egg-albumen solution (1:2) requires between 1:10,000 and 1:2,500. The test is therefore less than one-thirtieth as sensitive as in water.

d. Disappearance of iodin on contact with protein and urine. A chloroformic solution of iodin is rapidly decolorized on shaking with serum; egg-albumen, native, acid or alkali; or urine. Iodin in KI or elementary iodin added to serum, also yields no color when shaken with chloroform.

In all of these cases, however, there is a limitation to the amount of iodin that can be bound.

e. Quantity of iodin bound by serum. When 10 cc. of cat serum were shaken for an hour at 20°C. with 44 mgm. of iodin, dissolved in chloroform, 35.226 mgm. of the iodin was combined, equivalent to 3.523 mgm. of iodin per cubic centimeter of serum, or about 1: 3,000. This agrees well with the limit of sensitiveness noted under III *C*.

IV. DIALYSIS TEST FOR IODID, IODATE AND IODIN

Since it is impossible to determine iodid or other forms of iodin directly in the presence of soluble protein, recourse was had to dialysis through parchment or celloidin tubes, to remove the disturbing influence of the serum protein. This was found successful.

a. Speed and sensitiveness of dialysis test for iodid. With 4 cc. of NaI, 1: 50,000, dissolved in water, serum or egg-albumen, and diffused through parchment or celloidin against 4 cc. of water, the dialysate with the nitrite-acid-chloroform test was practically negative in twenty minutes, fairly positive in one or two hours. Even then, however, the dialysate was much weaker than the solution in the tube.

With stronger solutions (1: 10,000, in egg), the dialysate was negative in fifteen minutes, positive in forty-five minutes.

The diffusion occurred just as rapidly from the protein solutions as from water. The parchment and celloidin averaged about the same speed of dialysis.

The delicacy is therefore somewhat less than with the direct test in water, but is still sufficiently high; and is as high with protein containing solutions as with water. With dilution of 1: 50,000, the dialysis should be prolonged for two hours; with higher concentrations, it may be correspondingly shorter.

b. Dialysis test for iodate. A solution of KIO_3, 1: 50,000, was diffused through parchment and through celloidin against an equal volume of water, and tested after a day. The dialysate gave positive tests, although the inside fluid was still two or three times stronger than the dialysate.

c. Dialysis of free iodin. Iodin was liberated from NaI by

acid-nitrite and dialyzed through parchment and celloidin for half an hour. The celloidin dialysate contained an abundance of free iodin; the parchment dialysate much less, a part of the iodin combining with the parchment, as shown by blueing.

It is plain, therefore, that the presence and form of iodin compounds in serum may be demonstrated with great delicacy by simple dialysis. However, the method is not quantitative, at least in the form used, since the dialysis is incomplete, even with water.

The dialysis test is not applicable to urine, since the urinary constituents that bind iodin are dialyzable.

V. THE FATE OF IODID, IODATE AND IODIN, WHEN ADDED TO SERUM OR ALBUMEN IN VITRO

The dialysis test was utilized to determine what changes, if any, iodid, iodate or iodin undergo when added to serum or albumen. The experiments were conducted by placing the mixtures in parchment tubes, and dialyzing against water for the periods stated below. The dialysates were tested successively for free iodin by adding chloroform; for iodate by adding acid (since they always contained iodid); and for iodid by the further addition of $NaNO_2$. The results showed the following:

a. Iodid is not converted into iodate. NaI, added to albumen, 1:2,500, dialyzed three hours; no iodin or iodate; strong iodid.

NaI, added to serum, 1:50,000, dialyzed three days; no iodin or iodate; faint iodid.

b. Iodin forms iodid, but no iodate. A series of experiments was made on serum, adding iodin in chloroform, iodin in KI, and iodin in substance, and dialyzing for one hour, one day and three days. All the dialysates contained iodid, but no iodin nor iodate.

c. Iodid and iodate and excess of iodin dialyze from albumen without change. Undiluted egg-white is shaken with an excess Lugol's solution, and before any precipitate occurs, some KIO_3 solution is added. This is dialyzed through celloidin for a day; the dialysate is positive for iodin, iodate and iodid.

d. Iodin liberated in serum by nitrous acid is converted into iodid. Dog serum is mixed with NaI, 1:50,000, with the addition of acid and $NaNO_2$, and dialyzed through parchment for one hour and for three days; in both cases, the dialysate is negative for iodin and iodate, positive for iodid.

The results show that iodid and iodate are not attacked by serum; but that iodin is promptly bound, with the formation of iodid, unless an excess is present, in which case it can be demonstrated in the dialysate.

Proteins bind the iodin in their aromatic nuclei, analogous to the iodation of phenol:

$$C_6H_6O + 6I = C_6H_3I_2.0I + 3HI.$$

This accounts for the formation of the iodid. The iodin in the phenol-nucleus is held very firmly, so that it is not liberated by nitrous or iodic acid.

e. Proteins, urine and phenol prevent the conversion by alkali of iodin into iodid and iodate or hypoiodite. As is well known, alkalies bind iodin with the formation of hypoiodite, and later of iodate, according to the equation

$$(3)\quad 2\,NaOH + 2I = NaIO + NaI + H_2O$$
$$(4)\quad 3\,NaIO = NaIO_3 + 2NaI$$

or

$$(5)\quad 6\,NaOH + 6I = 5NaI + NaIO_3 + 3H_2O$$

The addition of acid regenerates iodin by reversing the reaction (5).

The reaction between iodin and alkali does not take place if there is present sufficient protein, urine or phenol to bind the iodin. If one adds iodin even to strongly alkaline solutions of these fluids, no iodin is liberated on subsequent acidulation (unless an excess of iodin had been added). It follows from this that the administration of free iodin could not produce any iodate or hypoiodite in the body; and that, once the iodin has been bound in the body, it cannot be regenerated by acid reaction.

This was confirmed by the direct experiment.

VI. IODIN ADMINISTERED BY STOMACH EXISTS IN THE BLOOD SOLELY AS IODID

Alcoholic solutions of iodin, about 7 per cent, with and without iodid, were administered to anesthetized dogs by stomach tube. The animals were bled after a time and a portion of the serum (about 15 cc.) was dialyzed through parchment. The dialysate was tested. The dialysis was continued, with daily changes of the water until the dialysate became free from iodid. The total iodin of the dialyzed serum was determined (by Dr. Collins) by fusion with nitrate-carbonate and titration with thiosulphate, and compared with the iodin content of another sample of the serum, fused directly.

The experiments were as follows:

Dog 8A, 18 kgm., 10 cc. tr. iodin, bled three hours later.
Dog 8B, 14 kgm., 25 cc. tr. iodin, bled three-quarters of an hour later.
Dog 14A, 14 kgm., 25 cc. alcoholic iodin (no KI), bled one hour later.
Dog 14B, 7.6 kgm., 25 cc. alcoholic iodin (no KI), bled one hour later.

Tests of dialysates

Dogs 8A and B, dialyzed one and one-half hours and three days: negative for iodin and iodate, positive for iodid.

Dogs 14A and B, gave the same results.

Determination of total iodin

	IODIN MGM. PER 100 CC. OF ORIGINAL SERUM	
	Original serum	Dialyzed serum
Dog 8A	2.114	0
	2.114	1.2684
Dog 8B	7.614	0
	7.190	0
Dog 14A	6.768	0.282
Dog 14B	5.960	0

In view of the absolutely negative results in most of the tests, it is probable that the two positive results were due to imperfect dialysis.

These experiments disposed in the negative of one phase of our problem; by showing that the administration of very large doses of free iodin (corresponding to 2 to 12 grams, or 30 to 180 grains for man) does not result in the presence of any iodin compounds in the blood, from which free iodin could be regenerated.

VII. INFLUENCE OF REACTION

Can iodin be liberated from an iodid-iodate mixture by the reaction of the body? It is clear from the preceding that no iodate can be formed from iodin in the presence of protein, urine, etc. (unless the iodin is added in such large quantities as need not be considered for the body). However, this leaves open the question whether the direct administration of iodate-iodid mixtures,[1] or of iodates in the presence of the sulphocyanate of the body, could give rise to the temporary liberation of iodin. This is essentially a question of the influence of the reaction (hydrogen-ion concentration) of the tissues. These problems were investigated by the use of "buffer mixtures."

a. Influence of reaction on disappearance of iodin. Phosphate buffer mixtures of Hp 7 to 8.2 are shaken with a very dilute iodin-chloroform solution, allowed to stand for a day, and the color of the chloroform compared:

No decolorization occurred with Hp 7; it was doubtful with 7.2; very slight with 7.4, and from here progressed with the alkalinity; but even at 8.2, the amount of iodin bound was extremely small. It is evident that the normal alkalinity of the blood can have but little influence on free iodin.

b. Influence of reaction on liberation of iodin. This was investigated by mixing 10 cc. of solutions of known hydrogen-ion concentration with:

(1) 0.5 cc. of 1 per cent NaI and 1 cc. of 0.1 per cent KIO_3.
(2) 0.6 cc. of 1 per cent NaI and 1 cc. of 0.1 per cent $NaNO_2$.
(3) 0.5 cc. of 4 per cent KIO_3 and 0.5 cc. of 4 per cent NaSCN.

Each of these should yield about the same quantity of iodin.

[1] According to Macciotta, 1916, Arch. farm. sper., 22, 3, potassium iodate, when given by stomach, is excreted exclusively as iodid. At some time, therefore, an iodate-iodid mixture must exist in the blood.

As acid solutions, there was used:

From Hp = 1 to 4: HCl, $\frac{N}{10}$ to $\frac{N}{10,000}$

From Hp = 4.49 to 8.4: Phosphate buffer mixture (furnished by Dr. Hanzlik).

The following results were obtained:

(1) NaI + KIO_3: Iodin liberated abundantly with Hp = about 2; much less with Hp = about 3; none with Hp = about 4 or 4.49 or lower.

(2) NaI + $NaNO_2$: Iodin liberated abundantly with Hp = about 2; practically none with Hp = about 3; none with Hp = about 4 or 4.49.

(3) KIO_3 + NaSCN: Iodin liberated abundantly with Hp = about 3; absent with Hp = about 4 or 4.49 or lower.

The results show clearly that the liberation of iodin from any of these mixtures requires acidity of over Hp = 4. This is much higher than can occur in the body, with the sole exception of the gastric juice (which may reach Hp = 1.77). Even the urine does not exceed 4.7. Saliva is at most 6.79. All other body fluids are below 7 (Michaelis: "Wasserstoffionenkonzentration"). Evidently, therefore, these reactions could liberate iodin only in the stomach, and nowhere else in the body.

SUMMARY AND CONCLUSIONS

(The numbers refer to the sections of the text)

I and II. The investigation was planned to determine (1) whether the administration of elementary iodin can result in the appearance of iodid and iodate in the blood or tissues; and (2) whether elementary iodin can be liberated from iodid and iodate, combined or separately, with the reaction (hydrogen-ion concentration) that is known to exist in the body.

III. Tests depending on the liberation of small quantities of iodin cannot be applied to protein-containing fluids, or to urine, since considerable iodin is immediately bound by the aromatic radicals. Under the conditions used, the amount of iodin bound by serum was about 1:3000. Larger quantities of iodin would, of course, be revealed; but the sensitiveness of the test is only about one-fiftieth to one-thirtieth of that in watery solution.

IV. The disturbing factor of proteins may be removed by dialyzing through parchment or celloidin. With dilute solutions, the dialysis should last two hours. This method is applicable, with sufficient delicacy, to iodids and iodates; and if collodion tubes are used, also to free iodin.

V. By the use of the dialysis method it was shown that neither iodid nor iodate undergo any change in serum. Free iodin is promptly bound, with the formation of iodid but no iodate is formed, even in the presence of alkali, so that subsequent acidulation does not liberate iodin (unless, of course, more iodin has been added than can be disposed of by the serum).

The statements hold also for other proteins, urine and phenol.

VI. Large doses of free iodin, administered by stomach, exist in the blood solely as iodid. The addition of acid to the blood does not regenerate iodin.

VII. No iodin is liberated from iodid-iodate or iodid-nitrite or iodate-sulphocyanid mixtures, until the acidity surpasses Hp = 4. It is therefore impossible that iodin should be liberated from iodid under any conditions existing in the body (excepting solely the gastric juice).

On the other hand, the alkalinity of the body is not sufficient to bind any noticeable quantity of free iodin—even in the absence of proteins and aromatic groups.

The questions that were proposed are therefore answered in the negative:

1. The blood, after the administration of free iodin, does not liberate iodin on acidulation, because the iodin is bound through the protein, and not through the alkali.

2. Free iodin cannot be liberated in the body, since all the conceivable reactions for the liberation of iodin from its compounds require much higher hydrogen-ion concentrations than exist anywhere in the body.

REFERENCES

(1) F. C. McLean: J. Amer. Med. Assoc., lxiii, 1382, 1914; Arch. Int. Med., xv, 92, 1915.

(2) R. Boehm and F. Berg: 1876, Arch. exp. Path. Pharm., v, 329.