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EFFECTS OF INORGANIC IODIDE ON THE INTERMEDIARY CARBOHYDRATE METABOLISM OF SURVIVING SHEEP THYROID SLICES *

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The rate of hormone formation by the thyroid *in vivo* is greatly influenced by acute alterations in the concentration of inorganic iodide in the extracellular fluid. In the rat (1-3) and in man (4, 5), increasing concentrations of iodide in the plasma are associated with increased thyroidal uptake of iodine until a critical concentration of iodide is reached. Beyond this range, the formation of hormone declines. A similar relationship between extracellular iodide concentration and organic iodinations occurs *in vitro* in slices of sheep thyroid (6), whole lobes of rat thyroid (7), and homogenates of rat salivary gland (8). This relative inhibition of thyroid hormone formation induced by high concentrations of iodide is often termed the Wolff-Chaikoff phenomenon, and has never been fully explained. In normal animals, such inhibition is transient (9). Rarely, however, prolonged treatment with iodine does cause goiter and myxedema in man (10), possibly because the inhibitory effect of iodide persists.

Recently, evidence has accumulated that the formation of hormone and oxidative metabolism in the thyroid are closely linked. First, the iodide-concentrating mechanism is dependent on phosphate bond energy (11, 12), and second, organic binding of thyroidal iodide appears to depend on electron transfers leading to generation of hydrogen peroxide (13-16). The present studies were designed to determine whether

changes in the rate of hormone formation induced by varying the availability of iodide to thyroid slices *in vitro* are associated with, or can be ascribed to, alterations of intermediary metabolism within the thyroid. A preliminary report of these studies has appeared (17).

METHODS

Preparation of slices. Thyroid glands were obtained from freshly killed sheep and were brought from the abattoir to the laboratory packed in ice. A single gland was used in each experiment. Slices were cut with a Stadie-Riggs microtome, washed twice in ice-cold physiological saline solution, weighed on a torsion balance, and placed in Warburg vessels containing 2 or 3 ml of ice-cold medium. Tissue weights were quite uniform within individual experiments, but varied between approximately 50 and 300 mg per flask in different experiments.

Preparation of media. The standard medium employed in control vessels was Krebs-Ringer-phosphate buffer (KRP), pH 7.4, with one-third the recommended concentration of CaCl_2 ¹ (18). Each milliliter of medium contained 1.5 or 2.0 mg of stable glucose, labeled with 0.5 to 1.5 μC ². Vessels were incubated at 38° C under 100% oxygen in a Warburg apparatus for from 60 to 200 minutes. CO_2 was absorbed by 0.2 ml of 10% KOH in a sidearm.

Media containing desired concentrations of iodide were prepared by isosmotic substitution of NaI for NaCl in KRP. Several factors, however, might cause the ultimate concentration of iodide in the medium to deviate from that produced by the addition of NaI. First, as judged by iodine analyses performed in 14 different preparations,³ the concentration of iodide in control media, representing contamination of the reagents

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¹ Modified Krebs-Ringer-phosphate buffer: NaCl, 0.132 M; KCl, 0.005 M; CaCl_2 , 0.0009 M; MgSO_4 , 0.0013 M; and phosphate buffer, 0.009 M.

² C^{14} -labeled glucose was obtained from the New England Nuclear Corporation, Boston, Mass.

³ Performed at Boston Medical Laboratory, Boston, Mass.

used, was considered negligible (average, 5.2 μg per 100 ml, or 4×10^{-7} M). Second, the quantity of iodide that might be leached from thyroid tissue during the incubation period was considered. Specimens of 9 separate thyroid glands were boiled in a test tube containing a small volume of distilled water. The entire contents of the tube were then placed in a dialysis sac and dialyzed overnight against distilled water at 4° C. The calculated content of dialyzable iodine in thyroids, based upon iodine analyses in the dialyzing solution, averaged 2.1 μg per 100 mg of tissue. In view of the subsequent demonstration that thyroid tissue contains substantial quantities of iodinated peptides that are slowly dialyzable (19–21), this value was undoubtedly an overestimation of the true concentration of inorganic iodide in these specimens. Even if this were not the case, flasks containing 100 mg of tissue and added iodide in a concentration of 1×10^{-5} M (1.27 μg per ml) would contain at least twice as much iodide as flasks containing tissue without added iodide. Furthermore, the glandular contribution of iodide would be insignificant in flasks in which media had been enriched to a concentration of 1×10^{-4} M or more. A third consideration was the ability of thyroid slices to perform iodinations and thereby to decrease the concentration of iodide in the medium. Here, too, this effect would almost always be negligible at iodide concentrations greater than 1×10^{-4} M, where percentile organic binding of iodide seldom exceeds 0.5% (6, 22).⁴ Finally, it is likely that any metabolic effects of iodide would be more closely related to concentration in the slice than to that in the medium, and that the activity of the iodide-concentrating mechanism would therefore influence the results obtained. Since slice/medium (S/M) iodide concentration gradients greater than one may exist at concentrations of iodide in the medium ranging up to 1×10^{-3} M (11), this factor was operative in most of the present studies. In them, no systematic attempt was made to correct for these factors. The iodide concentrations hereafter referred to therefore represent merely the concentration of iodide added to the medium, and flasks with no iodide will be termed "controls."

Measurements. Oxygen consumption was measured by standard manometric methods (18). C^{14}O_2 derived from labeled glucose was measured by methods previously described (15). In brief, these consisted of redistilling CO_2 from KOH into 0.5 M Hyamine, which was then transferred to vials containing liquid scintillator. Counting was carried out in a liquid scintillation counter, and counts in C^{14}O_2 were compared with counts in a sample of the initial medium, after correction for quenching. The recovery of counts from labeled glucose in vessels

⁴ That this effect might also be unimportant at lower concentrations of iodide is suggested by the work of Kondo (23). He found that, during active uptake of I^{131} by pig thyroid slices, iodide concentration in the medium remained constant or increased. Release of stable iodide from human and canine thyroid slices has been shown by Molnar, Albert, Keating, and Orvis (24).

incubated without tissue was not determined in the majority of experiments. C^{14} -labeled lactate in suspending media was estimated by ascending paper chromatography in butanol:acetic acid:water (100:12:88). That portion of the filter paper containing lactate was located by radioautography, excised, and placed in a vial of liquid scintillator for counting. Radioactivity in the lactate zone was compared with that in a sample of the initial medium applied to filter paper and counted under similar geometric conditions. Values for lactate production reported are the means of duplicate vessels; in no case did the value for an individual vessel differ from the reported mean by more than 7%. Concentrations of stable glucose were measured in duplicate by the Somogyi-Nelson method (25); control studies indicated that iodide concentrations in the medium as high as 1×10^{-1} M did not influence the glucose measurement. Total CO_2 production was determined by BaCO_3 precipitation, with care taken to prevent contamination with atmospheric CO_2 .

Statistical treatment. With but few exceptions, all results presented are the average of closely agreeing values obtained in duplicate or triplicate vessels. With some of the results, analysis of variance and tests of significance were performed as described by Snedecor (26). For comparison of results obtained in many thyroid glands, however, of markedly different metabolic activity, from animals whose age and history of iodide ingestion were unknown, it seemed preferable to use nonparametric methods, which do not assume a normal distribution of results. Therefore, the "sign test" was also employed (27, 28). The null hypothesis tested was that the number of experiments showing an increase in a given function (e.g., QO_2) at a given concentration of iodide would equal the number showing a decrease. The probability that the observed number of positive and negative differences occurred by chance was then taken from a standard table (28).

RESULTS

Oxygen consumption. In 41 experiments, oxygen consumption in control vessels averaged $28.6 \pm 8.7 \mu\text{l}$ per hour per 100 mg (mean \pm SD). The changes in QO_2 induced by varying concentrations of iodide in the medium are depicted in Figure 1, and their statistical significance is summarized in Table I. Low concentrations of iodide in the incubation medium (5×10^{-6} to 1×10^{-5} M) did not affect QO_2 with significant consistency. At iodide concentrations between 5×10^{-5} and 5×10^{-4} M, however, there was almost invariably an increase of QO_2 above control values (average, +17%), and this change was highly significant ($p < 0.001$) by the sign test (Table I). Significant stimulation of QO_2 above control values also occurred at iodide concentrations of

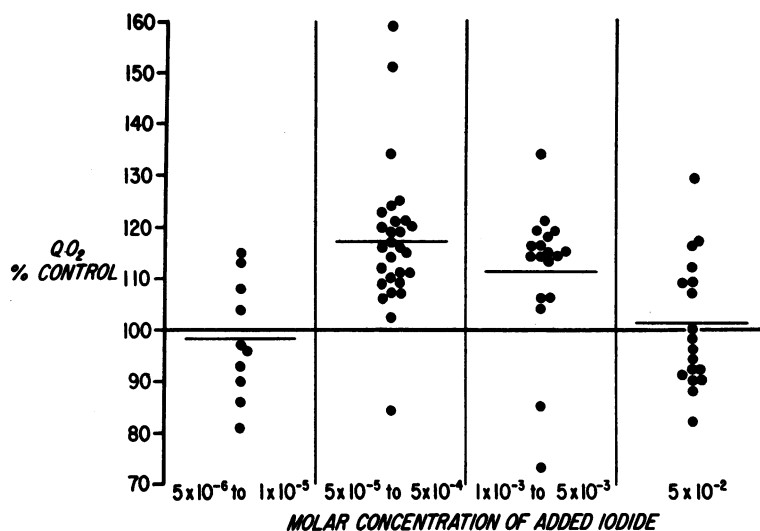


FIG. 1. EFFECTS OF INORGANIC IODIDE ON OXYGEN CONSUMPTION IN SHEEP THYROID SLICES. Within each concentration range, each point represents closely agreeing duplicate, triplicate, or quadruplicate results from a single thyroid gland.

1×10^{-3} to 5×10^{-3} M ($p < 0.001$), but was of smaller magnitude (average, +11%) and, when directly compared, was usually less than the stimulation occurring at 5×10^{-5} to 5×10^{-4} M. At the higher iodide concentration of 5×10^{-2} M, QO_2 's did not differ consistently from control values, but were consistently less than QO_2 's at the lower, stimulatory concentration ranges of iodide (5×10^{-5} to 5×10^{-4} M and 1×10^{-3} to 5×10^{-3} M).

In prolonged experiments, the rate of oxygen consumption declined slightly. In nine experiments 2 hours long or longer, QO_2 's during the

second hour averaged 7% less than during the first hour. In six experiments lasting 3 hours, QO_2 's during the third hour averaged 9% less than during the second hour. The proportionate decline in QO_2 with time was uninfluenced by the addition of iodide.

Experiments with uniformly-labeled glucose. In eight experiments, tissue was incubated in a standard concentration of stable glucose (2 mg per ml) labeled with glucose-U- C^{14} , and two standard concentrations of iodide (1×10^{-4} M and 5×10^{-2} M). Tissue weights in these experiments averaged 196 mg, and incubations were

TABLE I

Effect of added iodide on oxygen consumption by sheep thyroid slices seen in comparisons by the "sign test"

Iodide concentrations compared		n*	x†	p‡
A	B			
0	5×10^{-6} to 1×10^{-5}	10	4	>0.2
0	5×10^{-5} to 5×10^{-4}	28	26	<0.001
0	1×10^{-3} to 5×10^{-3}	18	16	<0.001
0	5×10^{-2}	18	8	>0.2
5×10^{-6} to 1×10^{-5}	5×10^{-5} to 5×10^{-4}	9	9	0.002
1×10^{-3} to 5×10^{-3}	5×10^{-5} to 5×10^{-4}	17	12	0.072
5×10^{-2}	5×10^{-5} to 5×10^{-4}	15	15	<0.001
5×10^{-2}	1×10^{-3} to 5×10^{-3}	10	9	0.011

* n = number of experiments in which comparisons of QO_2 's were made at the indicated concentrations of added iodide.

† x = number of experiments in which QO_2 was greater at iodide concentration B than at iodide concentration A.

‡ p = probability of difference between observed value of x and $x = n/2$.

TABLE II
Effect of inorganic iodide on $C^{14}O_2$ production from glucose-1- C^{14} and -6- C^{14} in sheep thyroid slices*

No. of exp'ts	Glucose-1- C^{14}				No. of exp'ts	Glucose-6- C^{14}			
	$C^{14}O_2$ production		Proportion of exp'ts with B > A \ddagger	p (sign test)		$C^{14}O_2$ production		Proportion of exp'ts with B > A \ddagger	p (sign test)
A \dagger	B - A \dagger	A \dagger			B - A \dagger	A \dagger	B - A \dagger		
	% of initial radioactivity/100 mg/hour					% of initial radioactivity/100 mg/hour			
Control (A) vs 5×10^{-6} to 1×10^{-5} M iodide (B)									
10	0.185 ± 0.111	+0.122 ± 0.036	8/10	0.055	5 \parallel	0.058 ± 0.019	+0.016 ± 0.005	4/4	0.062
Control (A) vs 1×10^{-4} to 1×10^{-3} M iodide (B)									
19	0.161 ± 0.074	+0.228 ± 0.049	19/19	0.001	11	0.074 ± 0.041	+0.072 ± 0.026	11/11	0.001
Control (A) vs 5×10^{-2} M iodide (B)									
9	0.163 ± 0.040	+0.131 ± 0.052	8/9	0.002	9 \parallel	0.073 ± 0.045	+0.031 ± 0.016	7/8	0.004
1×10^{-4} to 1×10^{-3} M iodide (A) vs 5×10^{-2} M iodide (B)									
6	0.414 ± 0.318	-0.106 ± 0.042	0/6	0.016	6	0.202 ± 0.130	-0.077 ± 0.020	0/6	0.016

* Standard conditions for these experiments were 3 mg glucose in 2 ml medium in each flask, and incubation time of 60 minutes.

\dagger $C^{14}O_2$ production at iodide concentration A (mean \pm SD).

\ddagger Difference between $C^{14}O_2$ production at iodide concentration B and that at concentration A (mean difference \pm SE of mean difference).

\S Figures represent no. of experiments where $C^{14}O_2$ production at concentration B exceeded that at concentration A/ no. of experiments where B \neq A. In the sign test, experiments where B = A are not included (27, 28).

\parallel In one experiment in this group, B = A.

allowed to proceed for an average of 165 minutes. Results obtained in this group of experiments are summarized in Table II; to facilitate comparisons among the various functions measured, data are expressed in micromoles.

In each of these eight experiments, changes in oxygen consumption were as noted above: stimulation at 10^{-4} M iodide and loss of stimulation at 5×10^{-2} M iodide. Changes in the generation of $C^{14}O_2$ roughly paralleled those in oxygen consumption.⁵ At 1×10^{-4} M iodide, however,

⁵ Hoskin has demonstrated that preparations of C^{14} -labeled glucose contain volatile radioactivity that is collected into alkali, even in the absence of tissue (29). Tissue-free blanks were measured in three of these experiments, and blank radioactivity was less than 0.008% of initially added radioactivity. In 24 subsequent experiments with glucose-U- C^{14} from the same source, not reported here, the amount of radioactivity recovered in KOH in vessels free of tissue (thermobarometers) averaged $0.009 \pm 0.002\%$ of medium radioactivity, and was uninfluenced by the addition of iodide. In the eight experiments reported here, radioactivity collected in alkali in vessels containing tissue and no added iodide averaged 0.46% of medium radioactivity (range, 0.21 to 0.88). Thus, it is unlikely that the true values for $C^{14}O_2$

there was in each experiment a greater percentile stimulation of $C^{14}O_2$ production than of CO_2 (mean increase, 71 against 19%). At 5×10^{-2} M iodide, some glands (experiments 37 and 67) displayed distinct stimulation of $C^{14}O_2$ production when compared with controls, while others showed essentially no change.

From a comparison of moles of oxygen consumed with the estimate of moles of glucose carbon converted to CO_2 , the proportion of oxygen consumption attributable to oxidation of exogenous glucose was calculated. This proportion was relatively small, averaging 12% in control flasks, 18% at 1×10^{-4} M iodide, and 14% at 5×10^{-2} M iodide. However, of the increase in oxygen consumption induced by 1×10^{-4} M iodide (mean increment, 0.25 μ moles per hour per 100 mg tissue), 48% could be ascribed to increased oxidation of exogenous glucose.

Assimilation of glucose (disappearance from the medium) was measured in six experiments.

production differed materially from those presented in Table II.

No consistent change induced by iodide could be detected. Errors in this estimation are relatively large, however, and the data do not exclude the possibility that small changes did occur.⁶ From the data in Table II, the proportion of assimilated glucose converted to CO₂ averaged 2.9% in control flasks, 5.9% in those containing 1 × 10⁻⁴ M iodide, and 3.7% in the presence of 5 × 10⁻² M iodide.

Respiratory quotients were determined in four experiments. Control values averaged 1.0, and no consistent changes were seen at either standard concentration of added iodide. Data with regard to the effects of iodide on the production of C¹⁴-labeled lactate yielded no evidence that the production of lactate was stimulated, and in two experiments (no. 37 and 67), iodide appeared to cause a decline in lactate production greater than the error of the method.

Analysis of variance of the data in Table II revealed that iodide produced significant changes only in oxygen consumption and in the production of C¹⁴O₂. With the criteria suggested by Snedecor (26), values for both functions in flasks containing 1 × 10⁻⁴ M iodide were significantly higher than values in control flasks, or in those containing 5 × 10⁻² M iodide. Differences between control flasks and those containing 5 × 10⁻² M iodide were not significant.

Experiments with glucose-1-C¹⁴ and -6-C¹⁴. Tissue-free blanks were not routinely incubated in experiments with either glucose-1-C¹⁴ or -6-C¹⁴; in two experiments, a blank with glucose-6-C¹⁴ was incubated, and 0.01% of medium radioactivity was found in KOH. In these studies with specifically labeled glucose, smaller amounts of tissue and shorter incubation times were employed than was the case in experiments with glucose-U-C¹⁴. Therefore, failure to correct for nonmetabolically generated radioactivity may have

⁶ The actual changes in medium glucose concentration during incubation in these experiments averaged about 25 mg per 100 ml, a difference easily measurable by the method used. As noted above, however, the mean increment in oxygen consumption induced by 1 × 10⁻⁴ M iodide was 0.25 μmoles per hour per 100 mg tissue. If this increment had been expended solely in the formation of CO₂ from an increase in assimilated glucose, the difference in final glucose concentration between control flasks and those at 1 × 10⁻⁴ M would have been only about 2.5 mg per 100 ml.

TABLE III
Effect of iodide on the metabolism of glucose-U-C¹⁴, oxygen consumption, respiratory quotient, and glucose assimilation in sheep thyroid slices

Exp't no.	O ₂ uptake μmoles/hour/100 mg		RQ		Net glucose assimilation		C ¹⁴ O ₂ production		Lactate-C ¹⁴ production	
	[0]*	[1 × 10 ⁻⁴]*	[0]	[1 × 10 ⁻⁴]	[0]	[1 × 10 ⁻⁴]	[0]	[1 × 10 ⁻⁴]	[0]	[1 × 10 ⁻⁴]
37	1.26	1.51	1.4	1.2	3.34	4.62	0.29	0.42	1.78	1.15
45	0.86	1.03	1.0	1.0	3.57	3.08	0.07	0.13		0.99
48	1.27	1.47	1.0	1.0	2.49	2.28	0.09	0.16		
52	0.72	0.81	0.8	1.4			0.09	0.12		
54	2.18	2.53			3.12	3.14	0.31	0.44		
63	1.49	1.72	0.8	0.9	5.40	4.67	0.05	0.08	1.93	2.14
67	1.32	1.99			4.63	4.20	0.22	0.68	0.80	0.65
71	1.43	1.52					0.16	0.22	1.50	1.31
Average	1.32	1.57	1.0	1.1	3.73	3.66	0.16	0.28		
Variance ratio "F"									5.47	1.93
									<0.025,	>0.01

* Numbers in brackets indicate molar concentration of added iodide in incubation medium.

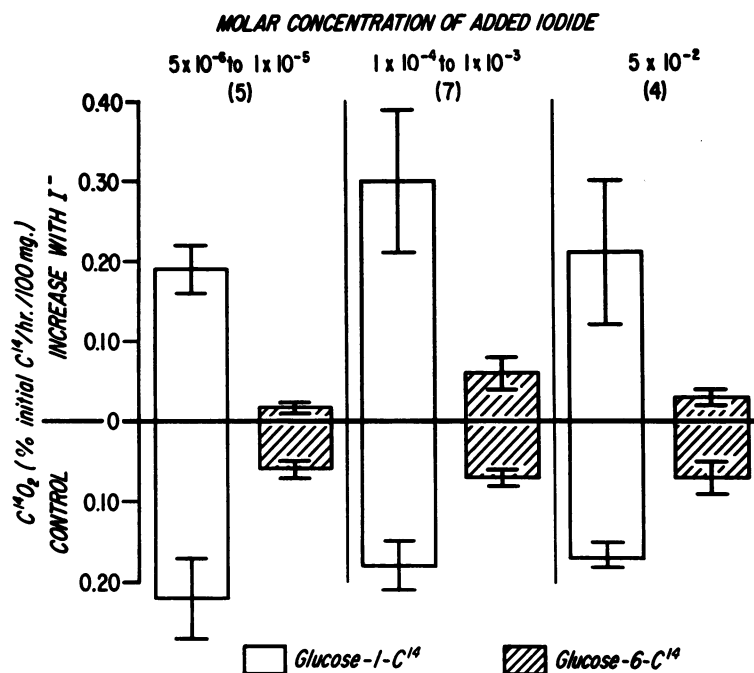


FIG. 2. COMPARATIVE EFFECTS OF IODIDE ON $C^{14}O_2$ PRODUCTION FROM GLUCOSE-1- C^{14} AND -6- C^{14} . Only experiments in which glucose-1- C^{14} and -6- C^{14} were used concurrently are depicted. In each experiment, each flask contained 3 mg glucose in 2 ml medium. $C^{14}O_2$ production in control flasks is indicated downward from the zero line (mean \pm SE); increases induced by the given concentrations of iodide are indicated upward from the zero line (mean difference \pm SE); number of experiments is in parentheses. Open columns represent results with glucose-1- C^{14} ; hatched columns represent results with glucose-6- C^{14} .

introduced significant errors in the determination of $C^{14}O_2$ production. Blank values may be assumed to be constant, however, among all the flasks in a single experiment; therefore, the amount of change in the generation of $C^{14}O_2$ induced by iodide in the medium can be accurately determined.

Results obtained in experiments with glucose-1- C^{14} and -6- C^{14} are shown in Table III. At the lowest range of iodide concentrations employed (5×10^{-6} to 1×10^{-5} M), generation of $C^{14}O_2$ from C-1- and C-6-labeled glucose was increased, and the changes were of borderline statistical significance by the sign test. At intermediate concentrations of iodide (1×10^{-4} to 1×10^{-3} M), and at the highest concentration of iodide employed (5×10^{-2} M), generation of $C^{14}O_2$ from both C-1- and C-6-labeled glucose was significantly increased. In experiments in which they were directly compared, effects of the intermediate

concentrations of iodide were greater than those of the highest concentration.⁷

In a number of instances, the effects of iodide on the generation of $C^{14}O_2$ from C-1- and C-6-labeled glucose were compared in the same experiment. Results obtained in these experiments are shown in Figure 2. In all cases, the increase in formation of $C^{14}O_2$ from glucose-1- C^{14} was far greater than from glucose-6- C^{14} .

Effect of thionamide derivatives. The effects of thiouracil and methimazole in concentrations known to inhibit organic iodinations (11) were assessed in four experiments (Figure 3). In vessels free of added iodide, these agents slightly,

⁷ It is not immediately apparent why $C^{14}O_2$ production was not consistently stimulated at an iodide concentration of 5×10^{-2} M in experiments using glucose-U- C^{14} , although such stimulation occurred rather consistently in experiments with glucose-1- C^{14} and -6- C^{14} . Variation between the individual thyroid glands studied may be the best explanation.

but consistently, inhibited the generation of $C^{14}O_2$ from glucose-1- C^{14} , but had no appreciable effect on QO_2 . Both thiouracil and methimazole, however, completely or almost completely inhibited the stimulatory response of these functions to added iodide, and a methimazole concentration of 5×10^{-3} M did not appear to be more effective than one of 1×10^{-4} M.

It may be presumed that both the stimulation of metabolism observed at moderate concentrations of iodide and the loss of stimulation at higher concentrations of iodide were more closely related to the concentration of iodide in the thyroid than to its concentration in the medium. In this regard, it has been clearly established that agents, such as methimazole, that inhibit organic binding *in vivo* concomitantly increase the concentration of iodide in the thyroid relative to that in the

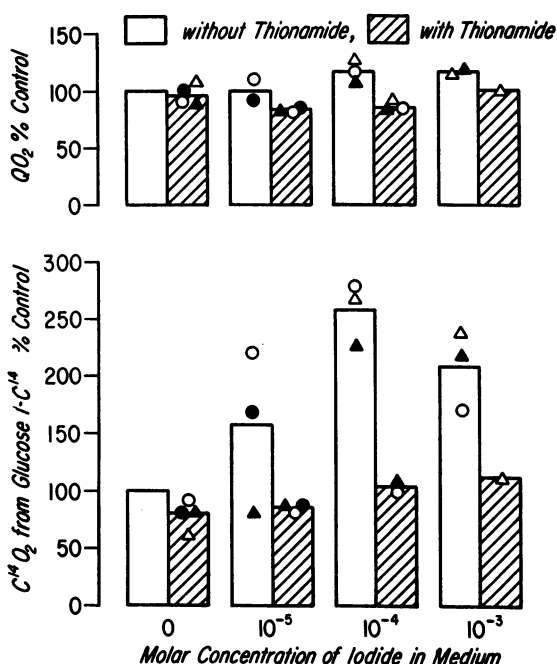


FIG. 3. THE EFFECT OF ANTITHYROIDAL AGENTS ON IODIDE-INDUCED STIMULATION OF OXYGEN CONSUMPTION AND $C^{14}O_2$ PRODUCTION FROM GLUCOSE-1- C^{14} IN SHEEP THYROID SLICES. Hatched columns represent flasks containing the antithyroidal agent. Each symbol represents results obtained with duplicate flasks in a single experiment. The agents used, and their concentrations, were: in experiment no. 14 (●), 2×10^{-3} M thiouracil; in no. 32 (△), 5×10^{-3} M methimazole; in no. 34 (○), 1×10^{-3} M methimazole; and in no. 35 (▲), 1×10^{-4} M methimazole.

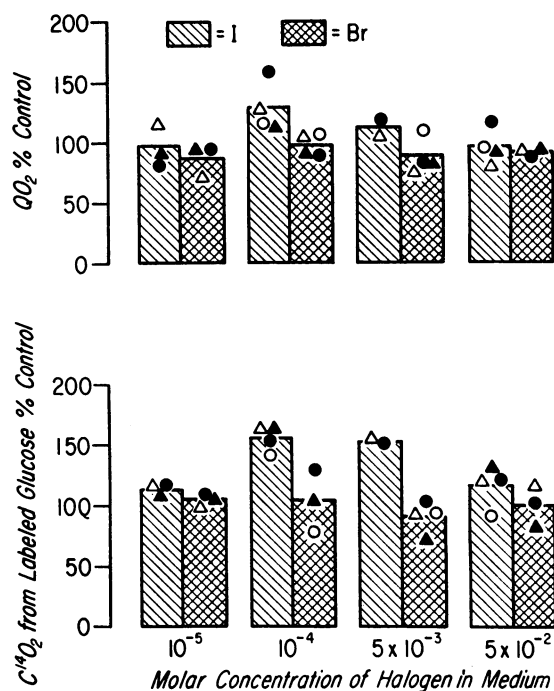


FIG. 4. THE EFFECT OF BROMIDE ON OXYGEN CONSUMPTION AND GLUCOSE OXIDATION IN SHEEP THYROID SLICES. Each symbol represents results obtained with duplicate flasks in a single experiment. In experiments 46 (△), 54 (○), and 56 (●), glucose-U- C^{14} was used; in experiment 59 (▲), glucose-1- C^{14} was used. Diagonally hatched columns represent flasks supplemented with iodide; cross-hatched columns represent flasks supplemented with bromide.

serum (30, 31).⁸ Thus, methimazole might have abolished the stimulatory effect of iodide by increasing the intrathyroidal concentration of iodide beyond the stimulatory range. The results of experiment 34, however, suggested that this was not the case. Here, in the absence of methimazole, stimulatory effects of iodide were evident at concentrations ranging between 1×10^{-5} M and 1×10^{-3} M (Figure 3). In the presence of methimazole, no stimulation of metabolism was evident at an iodide concentration of 1×10^{-5} M,

⁸ To test whether the same phenomenon occurs *in vitro*, slices were incubated in media containing 1×10^{-5} M iodide, with and without 1×10^{-4} M methimazole, and with tracer amounts of I^{131} . After an hour of incubation, I^{131} in slice and medium was partitioned into organic and inorganic moieties by precipitation with trichloroacetic acid. Without methimazole, 58% of the I^{131} was organically bound, and the S/M iodide gradient was 20. With methimazole, less than 1% of I^{131} was organically bound, and the S/M iodide gradient was 46.

TABLE IV
Effect of iodide on the metabolism of sheep liver and kidney slices

Exp't no.	Tissue	QO ₂			C ¹⁴ O ₂ production from glucose-C ¹⁴			
		Control	10 ⁻⁴ M I ^{-*}	5 × 10 ⁻² M I ⁻	Labeled carbon	Control	10 ⁻⁴ M I ⁻	5 × 10 ⁻² M I ⁻
		μl/hour/ 100 mg	% control	% control		% C ¹⁴ /hour/ 100 mg	% control	% control
12a	Kidney	133		93	C-1	1.0		73
					C-6	0.67		84
20a	Kidney	102		99	C-1	0.70		94
					C-6	0.70		68
73a	Kidney	166	96	93	C-U	0.42	90	83
76a	Kidney	132	105		C-U	0.56	98	
12b	Liver				C-1	0.058		71
					C-6	0.017		88
20b	Liver	12.3		92	C-1	0.072		96
					C-6	0.022		77
73b	Liver	23.4	81	55	C-U	0.030	103	93
74	Liver	32.3	110	85	C-U	0.013	96	91
75	Liver	32.8	92	96	C-U	0.100	88	114
76b	Liver	23.8	96		C-U	0.026	108	

* Concentration of iodide added to incubation medium.

and by direct measurement, S/M gradients for I¹³¹ were 30. Thus, methimazole, in vessels containing 1 × 10⁻⁵ M iodine, could not have increased intrathyroidal iodide to a concentration higher than that present in flasks containing 1 × 10⁻³ M iodide and no methimazole.

Effect of bromide. Four experiments were performed to test the effects of supplementing incubation media with bromide, rather than iodide (Figure 4). Since preparations of bromide may be contaminated with iodide, the iodide concentrations of media enriched with bromide were determined. These, however, did not differ significantly from those of simultaneously prepared KRP (KRP, 3.6 μg I per 100 ml; KRP and 5 × 10⁻² M bromide, 4.0 μg I per 100 ml). Bromide had no consistent effects either on QO₂, or on the production of C¹⁴O₂ from glucose-U-C¹⁴ (3 experiments) or glucose-1-C¹⁴ (1 experiment), even at the intermediate concentrations at which iodide was distinctly stimulatory.

Experiments with sheep liver and kidney slices. The concentration of iodide, 1 × 10⁻⁴ M, that most consistently stimulated oxidations in thyroid slices had no similar effect on slices of sheep liver and kidney (Table IV). At the higher concentration of 5 × 10⁻² M, however, iodide had a consistent inhibitory effect on QO₂ and decreased the

generation of C¹⁴O₂ from glucose-1-C¹⁴, -6-C¹⁴, and -U-C¹⁴.⁹ Tissue-free blanks were incubated in all the experiments with glucose-U-C¹⁴, and correction was made for blank radioactivity. Such blanks were not performed in the experiments with glucose-1-C¹⁴ and -6-C¹⁴.

DISCUSSION

The data presented here are generally in accord with earlier observations concerning the rate of oxygen consumption and glucose oxidation by sheep thyroid slices. They confirm previous studies indicating that the oxidation of exogenous glucose accounts for a relatively minor proportion (in the present studies, approximately 12%) of

⁹ If the intracellular concentration of iodide is the determinant of its metabolic effects, direct comparisons between the effects of standard extracellular concentrations of iodide on the thyroid and on other tissues that do not concentrate iodide are not possible. In liver and kidney, iodide is not actively concentrated (32, 33). Therefore, intracellular iodide concentrations in liver or kidney are probably lower than in thyroid when tissue slices from these respective organs are placed in media enriched with iodide at 1 × 10⁻⁴ M; at this concentration, thyroid slices maintain S/M gradients greater than unity (11). At a concentration of 5 × 10⁻² M iodide, however, thyroidal S/M gradients decline below unity (11), and intracellular iodide concentrations in thyroid, liver, and kidney are probably quite similar.

total oxygen consumption (34). This is consonant with the observation that the $\dot{Q}O_2$ of thyroid slices is well-maintained although no substrate is added to the medium (35-37). The endogenous substrate whose oxidation accounts for most of the oxygen consumed is, however, unknown. It has been reported that the concentration of glycogen in thyroid tissue is very low (38) and is insufficient to support observed rates of oxygen consumption (39). On the other hand, in agreement with the findings of Weiss (35), respiratory quotients in the present studies were near unity, suggesting that a major portion of the endogenous substrate is derived from carbohydrate. No data are presently available on the catabolism by thyroid slices of either endogenous lipid or the large stores of protein and protein-bound hexoses that the thyroid contains (40). The fate of the major proportion of assimilated glucose is unknown, since a very small proportion (3 to 6%) is converted to CO_2 , and only about 40% is converted to lactate.

The major finding of this study is that pronounced effects on certain aspects of thyroidal intermediary metabolism can be acutely induced by variations in extracellular iodide concentration. These effects are biphasic: oxygen consumption and conversion of exogenous glucose to CO_2 are stimulated by concentrations of iodide ranging up to approximately 1×10^{-4} M, a finding which has also been reported by Dumont (41), whereas at higher concentrations of iodide, these oxidative processes return toward normal.

Tentative conclusions can be drawn concerning the nature of the stimulatory metabolic effects of iodide observed in the present study. First, iodide caused increased oxygen consumption. In the absence of added iodide, oxidation of endogenous substrate in the thyroid appeared to account for much of the oxygen consumed. Although stimulatory concentrations of iodide increased the proportion of the oxygen consumed that was utilized for the metabolism of exogenous glucose, endogenous compounds of unknown nature remained the principal oxidative substrate, and their metabolism must have been increased. Furthermore, absence of significant change in respiratory quotients in association with increased $\dot{Q}O_2$ suggests that iodide changed the amount, rather than the nature, of the substrates concerned.

Evidence can be adduced concerning the pathways over which stimulatory concentrations of iodide produced their second major effect, increased production of CO_2 from exogenous glucose. It is clear that the quantity of exogenous glucose oxidized in the hexose monophosphate (HMP) shunt increased, in view of the greater increase that iodide induced in C-1 than in C-6 oxidation. It cannot be concluded, however, that iodide increased the proportion of glucose metabolized via the HMP shunt.¹⁰ Any factor that generally stimulates glucose metabolism will be manifested by a prompt increase in C-1 than in C-6 oxidation, since C-1 is immediately available for oxidation by the shunt, whereas C-6 must traverse a much longer series of reactions and be diluted in a greater number of intermediate pools before appearing as CO_2 . This is reflected in Dumont's finding that the ratio between $C^{14}O_2$ production from glucose-1- C^{14} and from glucose-6- C^{14} (the "C-1/C-6 ratio") declines progressively with time (44). This decline also occurs at stimulatory concentrations of iodide (22).

Several considerations indicate that the HMP shunt was not the sole metabolic pathway stimulated by iodide. These follow from the increase in the generation of $C^{14}O_2$ from glucose-6- C^{14} that iodide induced. This could have occurred merely as a result of increased formation of triose- C^{14} , without any alteration in its proportionate conversion to CO_2 . Had this been the case, then the percentile increase in formation of lactate- C^{14} should have equalled the percentile increase in formation of $C^{14}O_2$ from glucose-6- C^{14} . A change of this magnitude (an increase of over 50% at 1×10^{-4} M iodide) should readily have

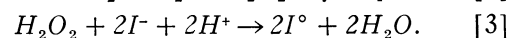
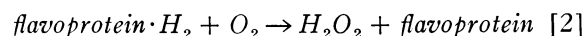
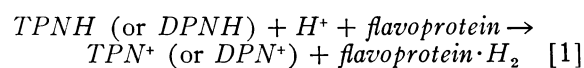
¹⁰ Katz and Wood have recently described a method for calculating the proportion of glucose metabolized via the HMP shunt by use of the fractional yields of $C^{14}O_2$ from assimilated C-1- and C-6-labeled glucose (42). Although yields of $C^{14}O_2$ from C-1- and C-6-labeled glucose were not precisely determined in the present studies, owing to failure to correct values for volatile contaminants in the glucose, this method was used to provide a first-order approximation of the percentile contribution of the HMP shunt to exogenous glucose metabolism in sheep thyroid slices. Calculations revealed this to be no more than a few per cent in control slices. Such low values are consonant with recent observations that the yields of C^{14} -labeled lactate from glucose-U- C^{14} or -1- C^{14} do not differ appreciably from the yields of labeled lactate obtained with glucose-6- C^{14} (43).

been detectable by the method employed for lactate measurement, and no such change occurred. Thus, the increased formation of $C^{14}O_2$ from glucose-6- C^{14} appears to have resulted from an increase in the proportionate conversion of triose to CO_2 . This increase could occur for several reasons. First, acetate- C^{14} derived from exogenous glucose may, in the presence of iodide, have been diluted to a lesser extent by acetate derived from unlabeled endogenous sources. This explanation seems inconsistent with the large increase in the oxidation of endogenous substrate that iodide induced. Second, a proportion of C^{14} reaching the 3-carbon pool may have been diverted from various synthetic reactions (e.g., formation of lipids and amino acids) to the formation of CO_2 . The present data do not exclude this possibility. Finally, iodide may have directly stimulated oxidative disposition of Krebs-cycle intermediates. Such an increase in Krebs-cycle oxidations could have resulted from an uncoupling of oxidative phosphorylation, such as iodide induces in rat liver mitochondria (45). Alternatively, it might reflect an increase in the availability of cofactors, such as oxidized pyridine nucleotides and flavins. This explanation seems especially attractive in view of the evidence of increased activity of both the HMP shunt and the Krebs cycle, as discussed above.

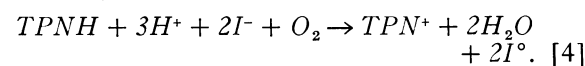
Several findings indicate that the stimulatory effects of moderate concentrations of iodide on thyroidal metabolism, whatever the pathways mediating them, are related to the effects of iodide on the rate of hormone synthesis. First, like the presently demonstrated increases in QO_2 and $C^{14}O_2$ production, total iodinations in sheep thyroid slices increase progressively as the iodide concentration in the medium is increased to approximately 1×10^{-4} M (6). Second, when organic iodinations are inhibited by methimazole or thiouracil, stimulation of metabolism by iodide is abolished. Furthermore, no metabolic stimulation is induced by bromide, a halogen that undergoes negligible organic binding (46-48). Finally, iodide induces no metabolic stimulation in liver and kidney, tissues that do not organically bind iodide. These findings suggest that the oxidation of iodide is linked to and somehow influences the rate of other thyroidal oxidations. The apparent paradox, that oxidation of one substrate,

i.e., iodide, can increase the rate of oxidation of other substrates, can be explained in several ways.

First, studies in thyroid homogenates and subcellular fractions have demonstrated a peroxidase capable of oxidizing iodide in the presence of H_2O_2 (13, 14). Evidence has recently been presented suggesting that the same enzyme may catalyze both the formation of H_2O_2 and subsequent peroxidations (49). The reactions involved in the generation of H_2O_2 probably occur according to the following sequence (15, 16):



Over-all:



If this is the correct mechanism, then it would follow from the present results that the rate of the over-all reaction depends on the concentration of iodide. In this way, oxidation of iodide could directly increase oxygen consumption and could indirectly stimulate other substrate oxidations by increasing the availability of oxidized pyridine nucleotides. Implicit in this argument, however, is the suggestion that the rate of peroxide formation is controlled by the rate of peroxide utilization, a situation not clearly shown to occur in biological systems.¹¹

One argument against the foregoing mechanism derives from stoichiometric considerations. In the postulated sequence of reactions, one mole of oxygen should be consumed for every two moles of iodide oxidized. An iodide concentration of 1×10^{-4} M in the medium, however, increased oxygen consumption by as much as $1.5 \mu\text{moles}$ per flask, although the flask contained only $0.2 \mu\text{mole}$ of iodide, of which only a small proportion would be expected to have undergone organic binding (6). This objection can be overcome, however,

¹¹As an alternative to this mechanism, it may be that reactions 1 and 2 proceed at a constant rate and that only reaction 3 is stimulated by iodide. If this were the case, and if, when the supply of iodide was limited, H_2O_2 were attacked by catalase, iodide would still stimulate net oxygen consumption, since the catalytic process returns one mole of oxygen for each two moles of H_2O_2 degraded. This mechanism per se, however, would not provide for increased substrate oxidations.

by postulation of a cyclic oxidation and reduction of iodine. This could come about in either of two ways. First, the thyroid appears to contain inhibitors of organic iodinations, such as ascorbic acid or glutathione (GSH) (15, 49, 50), that could reduce oxidized iodine before it is organically bound. If the inhibitor were GSH, the reaction (no. 5) would be: $2GSH + 2I^{\circ} \rightarrow GSSG + 2H^+ + 2I^-$. Since glutathione reductase is present in thyroid tissue (15, 51), the following reaction (no. 6) would also be expected: $GSSG + TPNH + H^+ \rightarrow 2GSH + TPN^+$.

A second, less direct mechanism for the reduction of iodine involves the deiodination of iodotyrosines by the thyroidal iodotyrosine deiodinase (52). This enzyme is TPNH-dependent (53, 54), and its operation presumably results in the generation of TPN from TPNH. In view of the recent demonstration that the thyroid contains free iodotyrosines that are rapidly turning over (55), a substantial recycling of iodide through iodotyrosines may occur.

Both mechanisms cited above for the reduction to iodide of an oxidized form of iodine provide for the generation of TPN from TPNH, and both might therefore be expected to enhance glucose oxidation. Indeed, addition of iodotyrosines to thyroid tissue has been shown to have this effect (38, 41, 56). If reactions 1 to 4 are also relevant, each mole of iodide cycled through an oxidation and reduction would consume $\frac{1}{2}$ mole of oxygen and would generate one mole of TPN. To the extent that extensive recycling of iodine occurs, therefore, especially when intrathyroidal iodide is moderately enhanced, a cascade of electron transfers would occur and would be associated with increases in both QO_2 and substrate oxidations. Quantitatively, this would be poorly reflected in net organic iodinations.

Finally, a third mechanism by which iodide may stimulate substrate oxidations has been suggested by de Groot and Davis, who demonstrated that an enzyme solubilized from a sheep-thyroid particulate fraction can peroxidize reduced pyridine nucleotides (57). The activity of this enzyme, and hence the generation of oxidized pyridine nucleotides, is directly related to iodide concentration, since iodide apparently protects the enzyme from degradation.

Although the foregoing mechanisms may ac-

count for the stimulatory effects of moderate concentrations of iodide on thyroidal oxidative metabolism, there remains to be considered the cause of the decline in or loss of stimulation occurring when concentrations of iodide are further increased. This biphasic metabolic response resembles the biphasic response of total organic iodinations to increasing concentrations of extracellular iodide, and thus provides a further suggestion that the oxidative metabolism of the thyroid gland and its rate of hormone formation are closely related. Both *in vitro* and *in vivo*, the thyroid responds to acute increases in extracellular iodide concentration with increasing organic iodinations. Beyond a critical concentration range, however, which in sheep slices is approximately 1×10^{-4} M (6), organic iodinations decline, although not necessarily to control levels. Two types of relationship between this so-called Wolff-Chaikoff effect and the biphasic effects of extracellular iodide on thyroidal oxidative metabolism can be envisioned. First, if the rate of iodide oxidation in some measure regulates oxidative intermediary metabolism in the thyroid, as suggested above, then declining oxidation of iodide during the Wolff-Chaikoff effect may explain the lessening of metabolic stimulation. It has been suggested that the Wolff-Chaikoff effect is due to complexing of oxidized iodine with iodide, in the presence of high concentrations of the latter, to form the triiodide ion I_3^- , which is incapable of carrying out iodination (8). This theory, still unproven, does not predicate a decreased initial oxidation of iodide. It does imply, however, that the TPNH-oxidizing reactions secondary to the recycling of iodide, which appear to be critical to the production of an appreciable metabolic effect, would be diminished or abolished.

An alternate relationship between the Wolff-Chaikoff effect and the decline in metabolic stimulation occurring at high concentrations of iodide also seems plausible. *In vitro*, high concentrations of iodide have been shown to inhibit two enzymes involved in oxidative metabolism: glucose-6-phosphate dehydrogenase (58) and fumarase (59). Such inhibition could account for the decrease in QO_2 and $C^{14}O_2$ production that high concentrations of iodide induced in slices of liver and kidney. Other related enzymes, such as aldolase (60) and lactic dehydrogenase (61), are

inhibited by molecular iodine, possibly through oxidation of sulfhydryl groups (62). If physiologically significant, such inhibition should be especially prone to occur in the thyroid. Thus, direct enzyme inhibition by iodide or iodine could explain both the declining phase of the intermediary metabolic response to varying concentrations of iodide and the secondary decline in the metabolically linked oxidation of iodide. At present, however, the data are insufficient to determine whether the effects of high concentrations of iodide are exerted primarily on iodide oxidation, or on intermediary metabolism, or whether, in fact, the two effects are interrelated.

SUMMARY

Enrichment of incubation media with increasing concentrations of inorganic iodide induced pronounced and biphasic alterations in the oxygen consumption and glucose metabolism of sheep thyroid slices. Moderate concentrations of iodide (up to 1×10^{-4} M) enhanced both QO_2 and the generation of $C^{14}O_2$ from glucose-U- C^{14} , -1- C^{14} , and -6- C^{14} . At high concentrations of added iodide (5×10^{-2} M), stimulation of these functions was diminished or absent.

The stimulatory effects of moderate concentrations of iodide appeared to be related to the oxidation or organic binding of increased quantities of iodine for the following reasons: 1) stimulatory effects were inhibited by methimazole and thiouracil, 2) bromide failed to stimulate thyroidal oxidations, and 3) no stimulation of oxidations was evident in slices of liver or kidney.

It is postulated that the stimulatory effects of iodide on glucose oxidation result from an enhanced oxidation of reduced coenzymes that may occur during both the oxidation of iodide and its subsequent reduction.

The loss of metabolic stimulation occurring at higher concentrations of iodide is of uncertain origin, but may be related, as either cause or effect, to the inhibition of thyroid hormone formation that high concentrations of thyroidal iodide acutely induce.

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