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Iodine Metabolism in the Thyroid: A Comparison of Whole and Sliced Lobes[1,2]

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The uptake of radioidodide from buffered solutions containing ^{131}NaI by thyroid tissue and its incorporation into protein has been reported by many investigators. The addition of thyrotropic hormone (TSH) to the media has caused an increase in the ratio between radioiodide in tissue and in media (T/M) of bovine thyroid glands incubated in the presence of propylthiouracil (1). The fraction of radioiodide that is bound to protein upon incubation with minced bovine thyroid has been demonstrated to be increased by TSH (2). The ratio of monoiodotyrosine to diiodotyrosine (MIT/DIT) formed *in vitro* appears to be related to the integrity of the thyroid tissue. High MIT/DIT ratios have been observed in thyroid homogenates (3), prefrozen or refrigerated thyroid slices (4) and in thyroids from rats exposed to excessive iodide (5). Rat thyroid tissue in the form of whole or sliced thyroid lobes has been employed in numerous studies or thyroid function. Because the thyroid lobe of the rat is small and slicing of the lobe technically difficult, the use of whole lobes for *in vitro* incubation has been preferred by some. On the other hand, the problems of penetration might be more conveniently approached if the lobes are sliced. A series of studies has been carried out employing whole or sliced lobes from rats maintained on an iodine deficient diet in order to evaluate the results under these two circumstances. These results are compared with those obtained from the incubation of whole thyroid lobes from rats on a stock laboratory diet.

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[2]A preliminary report of this work has been published (*Federation Proc.* 23:203, 1964).

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METHODS

All experiments were carried out with thyroid lobes from male rats. Two groups were employed: those fed a commercial low iodine diet (Nutritional Biochemicals, to be referred to subsequently as L.I.D.) for a period of 10 to 15 days prior to sacrifice and a second group maintained on a stock laboratory diet (to be referred to subsequently as high iodine diet or H.I.D.). Thyroid lobes from two rats constituted an experimental pair. The lobes were crossmatched so that every flask contained two thyroid lobes, one from each of the rats. One flask of each pair also contained 20 mu/ml TSH (Thytropar, Armour Pharmaceutical Co.). When sliced lobes were employed, the lobes were placed on a glass plate moistened with chilled buffer and sliced with a razor. Following this, the sliced lobes were processed in paired flasks in the same manner as were whole lobes. All thyroids were weighed prior to transfer to 10 ml Erlenmeyer flasks for incubation. Incubation was carried out for three hours in a phosphate buffer, pH 7.4 as previously described (2). The buffer contained 1 to 15μc ^{131}I. The inorganic iodide content of the buffer was found to be 1×10^{-7}M. With the addition of inorganic iodide, buffer was prepared having an iodide content of 10^{-6}, 10^{-5} and 10^{-4}M and incubations were carried out at all four levels of iodide concentration. Buffer volume was adjusted to tissue weight according to the following schedule; 30 mg/1 ml, 45 mg/1.5 ml, 60 mg/2 ml, 75 mg/2.5 ml. Following incubation, the thyroids were rinsed briefly in chilled buffer and transferred to tubes containing 1 ml veronal buffer pH 8.6, supplemented with 1×10^{-3} M thiouracil for homogenization (6). An aliquot of the homogenate was counted in a well scintillation counter. Thyroidal ^{131}I uptake was determined as the per cent of buffer radioiodide concentrated per mg thyroid. The separation of thyroidal iodide ^{131}I from protein bound radioiodide (^{131}PBI) in the homogenate was accomplished by filter paper chromatography. The remainder of the homogenate was hydrolyzed by the addition of pancreatin. Chromatography of the hydrolysate as well as of homogenate samples was carried out in an ascending fashion in butanol:acetic acid:water (78:5:17). All samples were chromatographed in duplicate. The chromatograms were scanned in an automatic strip scanning counter and the areas of radioactivity measured by planimetry of the counting record. ^{131}PBI was calculated as the per cent ^{131}I on the chromatogram remaining at the origin. The ^{131}MIT/^{131}DIT ratio was calculated as the ratio of the area corresponding to each of these two labeled amino acids on the counting record of the hydrolysate chromatograms.

RESULTS

The results observed for iodide ^{131}I uptake are presented in Table I. Mean uptake by L.I.D. slices was consistently less ($p < .001$) than that of L.I.D. whole lobes at all four levels of buffer iodide employed. As buffer iodide concentration increased the per cent iodide ^{131}I uptake diminished in L.I.D. slices and H.I.D. lobes. The addition of TSH to the buffer failed to influence iodide ^{131}I uptake by the thyroid except in H.I.D. whole lobe incubations at 10^{-7} and 10^{-6}M buffer iodide ($p < .05$ and $p < .01$), respectively. Data for stable iodide uptake calculated

TABLE I

THYROIDAL UPTAKE OF ^{131}IODIDE

(Per cent buffer ^{131}iodide/mg of thyroid)

Buffer Iodide Concentration	*TSH mμ/ml*	*L.I.D. Slices*	*L.I.D. Whole Lobes*	*H.I.D. Whole Lobes*
10^{-7}M (6)‡	0	1.15 ± .11*	1.47 ± .18	1.21 ± .10
	20	.99 ± .09	1.36 ± .19	1.49 ± .15
10^{-6}M (8)	0	1.04 ± .14	1.74 ± .19	1.06 ± .09
	20	.83 ± .04	1.90 ± .24	1.25 ± .11
10^{-5}M (7)	0	.89 ± .12	1.70 ± .19	.94 ± .09
	20	.87 ± .10	1.71 ± .20	1.0 ± .09
10^{-4}M (6)	0	.36 ± .09	1.37 ± .05	.78 ± .06
	20	.33 ± .05	1.36 ± .09	.66 ± .06

*Mean s.e.
‡The numbers in the brackets indicate the number of experiments.

TABLE II

THYROIDAL ^{127}IODIDE UPTAKE FROM BUFFER

(mμg iodide/mg of thyroid)

Buffer Iodide Concentration	*TSH mμ/ml*	*L.I.D. Slices*	*L.I.D. Whole Lobes*	*H.I.D. Whole Lobes*
10^{-7}M	0	.155 ± .014*	.233 ± .010	.162 ± .019
	20	.137 ± .016	.246 ± .016	.203 ± .021
10^{-6}M	0	1.65 ± .207	3.15 ± .08	1.48 ± .19
	20	1.47 ± .240	3.06 ± .11	1.76 ± .14
10^{-5}M	0	11.99 ± 1.52	26.98 ± 1.15	14.52 ± .90
	20	12.0 ± 1.15	27.06 ± .95	16.14 ± 1.33
10^{-4}M	0	53.82 ± 10.5	198.05 ± 26.8	99.33 ± 8.3
	20	44.86 ± 6.7	197.7 ± 34.1	84.4 ± 9.06

*Mean ± s.e.
Thyroidal ^{127}I uptake = % buffer-iodide-^{131}I/mg of thyroid × V × C
V = the volume of buffer employed
C = iodide concentration of the buffer

from iodide ^{131}I uptake and the buffer iodide concentration are presented in Table II. The results are expressed in millimicrograms (mμg) of iodide ^{127}I uptake/mg of thyroid. Iodide ^{127}I uptake by whole thyroid lobes from rats maintained on an iodine deficient diet was greater at every buffer iodide level than that of L.I.D. sliced lobes as well as for H.I.D. whole lobes. In the absence of TSH, uptake by L.I.D. sliced lobes was similar to that attained by H.I.D. whole lobes at 10^{-7}, 10^{-6}, and 10^{-5}M buffer iodide. Stable iodide uptake by H.I.D. whole lobes was significantly greater ($p<.01$) than that of L.I.D. slices at 10^{-4}M buffer iodide.

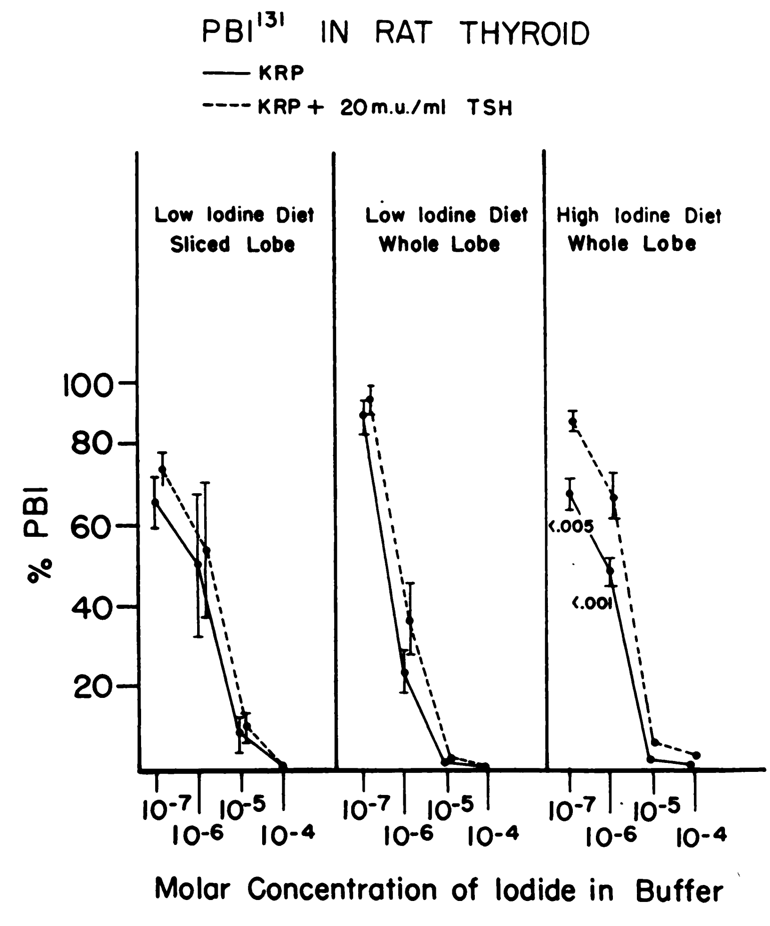

Fig. 1. The mean values for the fraction of thyroid ^{131}I bound to protein are expressed as the per cent ^{131}PBI. The per cent ^{131}PBI is plotted on the *ordinate* and buffer iodide concentration on the *abscissa*. The vertical bars about each point represent the standard error of the mean. TSH produced a significant change in ^{131}PBI only in whole thyroid lobes from rats on a high iodine diet at 10^{-7} and 10^{-6} M buffer iodide and the level of significance is indicated beneath these points.

The results for the determination of the fraction of thyroidal ^{131}I bound to protein (^{131}PBI) are summarized in Fig. 1. In thyroids from rats fed an iodine deficient diet, ^{131}PBI was significantly higher (p.<025) in whole lobes as compared to sliced lobes at 10^{-7}M buffer iodide. The mean value for ^{131}PBI in L.I.D. sliced lobes and whole lobes was not significantly different at 10^{-6}M buffer iodide. The fall in ^{131}PBI between 10^{-7} and 10^{-6}M buffer iodide in L.I.D. whole lobes was significant ($p<.001$), whereas the difference in ^{131}PBI for L.I.D. sliced lobes at the same buffer iodide concentrations was not. Thus, it would appear that ^{131}PBI formation in whole lobe is more sensitive to a rising buffer iodide level than sliced lobes. At 10^{-5}M the mean value for ^{131}PBI of L.I.D. sliced lobes was 8.7 per cent, that for whole lobes less than 1 per cent, but the difference was not significant. TSH had no apparent effect on ^{131}PBI in L.I.D. sliced lobes and whole lobes, but did increase the ^{131}PBI in H.I.D. whole lobes at 10^{-7} and 10^{-6}M buffer iodide ($p<.005$ and $p<.001$).

The fraction of ^{131}I in the thyroid digests present as mono and diiodotyrosine was determined by chromatography. From this data, the ratio of $^{131}MIT/^{131}DIT$– ^{131}I was calculated and the results are presented in Table III. Since labeled iodotyrosines were present in trace levels only at 10^{-5} and 10^{-4}M buffer iodide, values for $^{131}MIT/^{131}DIT$– have been calculated for the lower two buffer iodide levels only. The $^{131}MIT/^{131}DIT$– ratio was much higher in L.I.D. slices than in whole lobes at 10^{-7}M buffer iodide. As buffer iodide increased, the mean value for labeled MIT/DIT in L.I.D. whole lobes increased from 1.93 ± .26 to 3.22 ± .52. However, this change was not statistically significant.

DISCUSSION

The difference in iodide ^{131}I uptake in whole thyroid lobes from animals on low and high iodine diets is not unexpected. The modifying effect of slicing on

TABLE III

$M^{131}IT/D^{131}IT$

IN THYROID HYDROLYSATE

M Buffer Iodide Level	*TSH mμ/ml*	*L.I.D. Slices*	*L.I.D. Whole Lobes*	*H.I.D. Whole Lobes*
10^{-7}M	0	3.54 ± .47‡	1.93 ± .26	2.0 ± .26
	20	3.31 ± .5	1.72 ± .04	1.4 ± .03
10^{-6}M	0	4.3 ± 1.28	3.22 ± .52	1.98 ± .42
	20	3.95 ± 1.06	3.1 ± .99	1.49 ± .27

‡Mean ± s.e.

iodide ^{131}I uptake is consistent with other observations on altered thyroid tissue (2, 3, 4). In the studies reported here, organic binding by the thyroids was not blocked by propylthiouracil, and iodide ^{131}I uptake under these circumstances may not relate to the results reported by others for tissue/medium iodide ratios. Although it seems logical to attribute the difference in iodide ^{131}I uptake between whole and sliced thyroid lobes to an alteration in trapping ability, this point has not directly been examined as yet.

The increase in iodide ^{131}I uptake induced by TSH in whole thyroid lobes from rats maintined on a high iodine diet was similar to that observed by us in minced beef thyroid (2). The failure of a TSH response in both whole and sliced thyroid lobes from rats fed an iodine deficient diet was surprising. A biphasic effect of TSH on thyroid/serum iodide ^{131}I ratios in rats has been reported by Halmi and coworkers (6). In studies carried out under *in vitro* conditions by the same authors, the thyroid/medium iodide ^{131}I ratio was decreased in thyroids from rats on a high iodine diet when TSH was added to the buffer. When thyroids from goitrogen treated rats were employed, TSH had no effect on the thyroid/medium iodide ^{131}I ratio. From these observations it was concluded that the lack of stimulation by TSH could not be attributed to an increased release of inorganic iodine from thyroglobulin. Powell, Rahman and Deiss have recently presented evidence that in the presence of TSH, radioiodide uptake by bovine thyroid slices was increased shortly after incubation began and before a significant release of stable iodide by the slice was noted (7). In the present experiments, if the lack of response of iodide ^{131}I uptake to TSH noted in the L.I.D. thyroids was due to iodide release from the thyroids, this effect should have been even more pronounced in H.I.D. lobes. Quite the contrary was observed. Whole lobes from rats maintained on a stock laboratory diet demonstrated a response of iodide ^{131}I uptake to TSH at 10^{-7} and 10^{-6}M buffer iodide.

In the increased iodide concentrating ability of the thyroid which develops in iodine deficiency the exact role played by iodide depletion itself as compared to the thyroid-pituitary feedback mechanism is difficult to determine. It is conceivable that in rats maintained on an iodine deficient diet, some aspect of the iodide cycle may be under maximal stimulation by endogenous TSH, and unresponsive to TSH when studied under *in vitro* conditions. The present experiments do not preclude the possibility that the concentration of TSH chosen was relatively ineffective as compared to the endogenous thytropin secretion to which the thyroids have been exposed.

The inhibitory effect of high iodide levels on the binding of iodide to protein has been demonstrated both *in vivo* and *in vitro* (8, 9, 10). In the present experiments, an increase in buffer iodide was followed by a decrease in ^{131}PBI in the thyroid. However, the magnitude of the fall was not the same in all experiments. The decrease in ^{131}PBI observed between 10^{-7} and 10^{-6}M buffer iodide in L.I.D. whole lobes was statistically significant, while that observed in L.I.D. sliced lobes was not. The explanation for this difference may be found in the stable iodide uptake at 10^{-6}M buffer iodide: the mean for L.I.D. lobes was 1.65

$m\mu g$ iodide/mg of thyroid, that for L.I.D. whole lobes was 3.15 $m\mu g$/mg of thyroid. At 10^{-5}M buffer iodide the ^{131}PBI was slightly higher in L.I.D. sliced lobes (8.6%) than in either L.I.D. or H.I.D. whole lobes. It has been estimated that organification of iodide is almost completely inhibited at thyroidal iodide concentrations greater than 2 mg/gram of thyroid (9). Based upon our calculations of stable iodide uptake, this level of thyroid iodide concentration (20 mg/mg)[1] was exceeded in L.I.D. whole lobes at 10^{-5}M and by all experimental groups at 10^{-4}M buffer iodide. From the foregoing, we would conclude that the reduced concentrating ability of the sliced lobe as compared to the whole lobe has resulted in higher values for the fraction of thyroidal ^{131}I bound to protein at certain critical buffer iodide levels. The data for the ratio of ^{131}MIT/^{131}DIT tend to support this conclusion. In L.I.D. sliced lobes, the labeled iodotyrosine ratio was high and this finding is consistent with other observations on altered thyroid tissue (2, 3, 4). In L.I.D. whole lobes the mean value for ^{131}MIT/^{131}DIT at 10^{-7}M buffer iodide was 1.19, but increased to a mean of 3.2 at 10^{-6}M. Although this difference was not statistically significant, the value for the student T test fell just above the 5 per cent level. Galton and Pitt-Rivers have found the iodotyrisine ratio to increase with iodide inhibition and the trend of our data is in agreement with this finding (5).

SUMMARY

Thyroids from rats fed a low iodine diet were incubated as whole lobes and sliced lobes in buffer containing iodide ^{131}I. Total uptake of iodide ^{131}I and the fraction of thyroidal ^{131}I bound to protein was determined. The distribution of labeled iodoamino acids was observed following chromatography of pancreatic digest of the thyroid. The values for iodide ^{131}I uptake and protein bound ^{131}I were higher and the ratio of monoiodotyrosine ^{131}I/diiodotyrosine-^{131}I lower in whole lobes as compared to sliced thyroid lobes. When the iodide concentration of the buffer was increased, whole thyroid lobes from rats fed an iodine deficient diet were more sensitive to the inhibitory effect of iodide on protein binding of iodine than was true for sliced lobes. This difference in sensitivity appeared to be related to the greater quantity of stable iodide concentrated by whole thyroid lobes. Neither whole lobes or sliced thyroid lobes from rats maintained on an iodine deficient diet showed any response to TSH. Whole thyroid lobes from rats on a stock laboratory diet demonstrated an increase in iodide ^{131}I uptake and the fraction of ^{131}I bound to protein when incubated in the presence of TSH. It is suggested that in rats on a low iodine diet, endogenous TSH secretion has effected maximal stimulation of some step in the intrathyroidal iodine cycle so that the gland is insensitive to TSH in the buffer.

[1]The Iodine content of the Remington diet, as determined in this laboratory, was 4-7μg/100 gm.

The iodine content of the stock laboratory diet was 210 μg/100 gm.

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