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# Iodine Metabolism in Cultured Mouse Thyroid Lobes[1] (38406)

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Many investigators have used rat or mouse thyroid lobes for *in vitro* studies of iodine metabolism. The value of these experiments has been limited in that tissue viability is poorly maintained and only rarely does one recover significant quantities of labeled iodothyronines from the incubated lobes (1, 2). More recently several laboratories have applied organ culture techniques to the thyroid. Using this method, Nataf and Chaikoff (3) and Beck *et al.* (4) found significant quantities of labeled iodothyronines in rat thyroid lobes incubated in radioiodine-supplemented medium for periods up to 24 hr. Similarly, studies in this laboratory with lamb thyroid slices in an organ culture system demonstrated a remarkable maintenance of structural integrity and the ability to form significant quantities of labeled hormone, even after many days of culture (5). However, we were unable to demonstrate a consistent response for iodine labeled metabolites when Thyroid Stimulating Hormone (TSH) was added to the medium.

The present experiments were undertaken with the expectations that thyroid lobes from mice fed a low iodine diet might demonstrate a greater sensitivity of iodine metabolism to *in vitro* TSH and yet because of their small size, retain functional integrity in culture for more than just a few hours. Our results confirm the utility of organ culture methodology in assessing iodine metabolism in mouse thyroid lobes. Further, the extreme sensitivity of the mouse thyroid lobe to *in vitro* TSH under the experimental conditions we employed suggest a wide applicability of this technique to a variety of metabolic studies of the thyroid.

*Methods. General culture conditions.* Female Sprague–Dawley mice, 3 weeks old, were maintained on a low iodine diet (Nutritional Biochemical) and deionized water for a period of 2 weeks prior to sacrifice. Following exsanguination under ether anesthesia, trachei from two mice with the thyroid lobes attached were removed, stripped of connective tissue and placed in a 10 × 35 mm plastic culture dish (Falcon). They were positioned on the middle portion of a rayon strip, resting on a stainless steel grid, thus, elevating the strip slightly above the surface of the medium. Both ends of the strip were immersed into the medium (McCoys 5A supplemented with 20% v/v heat inactivated serum). The serum for these experiments was obtained from a lamb treated with thyroxine in order to suppress endogenous TSH production. Incubations were carried out in an atmosphere of 95% oxygen, 5% carbon dioxide at 32° for periods up to 24 hr. All experiments were performed in triplicate.

*Radioiodine incorporation experiments.* Thyroid lobes were incubated with 50 $\mu$Ci $^{131}$I/ml of medium at a pH of 8.00 ± 0.20. Afterwards, they were homogenized and subjected to hydrolysis using pronase (6). The hydrolysates were chromatographed in butanol-acetic acid and in either butanol–dioxane–ammonia or butanol–ethanol–ammonia systems. Radioactive compounds of interest were quantitated by means of scanning in an automatic chromatogram scanner or by counting portions of the chromatograms located by radio-autography. In experiments where the effect of TSH on incorporation was studied, 15 $\mu$g of thyroxine was administered intraperitoneally to each mouse followed by four days of a thyroxine supplemented, low iodine diet.

*Radioiodine release experiments.* Mice which had been fed a low iodine diet for 2 weeks were injected intraperitoneally with 50 $\mu$Ci of carrier free $^{131}$I 2 hr prior to sacrifice. In these experi-

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ments the incubating medium was supplemented with 1 m*M* propylthiouracil in order to prevent recycling of radioiodine in the thyroid. The pH of the medium was adjusted and maintained at 7.40 ± 0.05. At various intervals, the tissue and media were harvested and homogenized. The resulting homogenates and aliquots of media were chromatographed without prior hydrolysis. The fraction of total radioactivity which was recovered as inorganic iodide and iodothyronines in both medium and homogenate was calculated for each culture dish. The results for cultures in which medium was supplemented with TSH were expressed as percent of the controls in each experiment (7).

*Transport experiments*. Thyroid lobes were incubated for 24 hours in a medium containing 1 $\mu$Ci/ml of carrier free $^{131}I$, $10^{-5}$ mole NaI and 2 mmole methimazole. TSH (50 mU/ml) or $10^{-5}$ mole $KClO_4$ were added to the medium in certain experiments. At the end of the incubation, the lobes were removed, washed quickly and homogenized in 0.85% NaCl. The radioactivity and protein content (8) of a homogenate aliquot was then determined in order to calculate cpm/mg of thyroid protein.

*Results*. Initial experiments were directed towards establishing optimal conditions of incubation. Two important variables were, (a) addition of serum to the medium and, (b) the pH of the incubating medium. As depicted in Table I, there was almost a twofold increase in the incorporation of $^{131}I$ into iodothyronines and iodotyrosines and a decrease in the MIT/DIT ratio when the concentration of lamb serum was increased to 20% v/v. When the medium pH was increased from 7.40 to 8.00 labeled hormone formation was doubled but there was no change in the fraction of thyroidal radioiodine recovered as iodotyrosine.

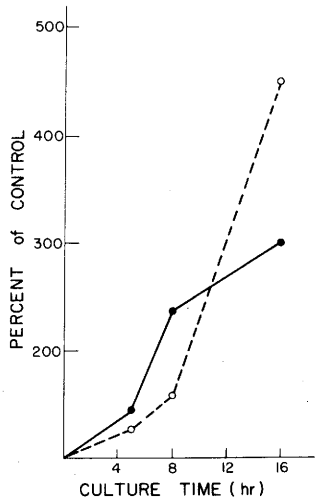


FIG. 1. Time course of the TSH effect on the release of iodide (●) and iodothyronine (○) by cultured mouse thyroid lobes.

The results of a typical iodine incorporation experiment in which TSH (20 mU/ml) was added to the medium is shown in Table II. Radioiodine uptake by the lobes was doubled and the quantity of thyroidal $^{131}I$ recovered as inorganic iodide was markedly decreased. The $^{131}$MIT/DIT ratio fell from 1.56 to 1.0 and iodothyronine formation increased nearly eightfold.

Figure 1 shows the time course of the effect of TSH on the release of iodide and iodothyronine. Note that at 8 hr of culture with a TSH concentration of 2 mU/ml, the labeled iodothyronine release lagged behind that of $^{131}I$. However, by the 16th hour, iodothyronine release surpassed that of iodide. Disparate iodothyronine and

TABLE I. Effect of Medium Serum Levels on the Uptake and Distribution of $^{131}I$ by Cultured Mouse Thyroid Lobes.[a]

| Percent (v/v) lamb serum added to media | Distribution of $^{131}I$ in hydrolysates of mouse thyroid lobes (%) Total $^{131}I$) | | | | | Percent $^{131}I$ uptake by mouse lobes |
|---|---|---|---|---|---|---|
| | MIT and DIT | $I^-$ | $T^4$ | $T^3$ | MIT/ DIT | |
| 0 | 23.17 | 60.71 | 8.85 | 2.58 | 1.00 | 24.79 |
| 10 | 37.74 | 41.55 | 12.85 | 3.13 | 1.04 | 28.00 |
| 15 | 49.92 | 27.69 | 14.57 | 2.56 | 1.08 | 21.57 |
| 20 | 47.56 | 24.20 | 16.77 | 3.38 | 0.83 | 22.92 |

[a] Cultures were for 16 hr at pH 8.0.

TABLE II. Effect of TSH on the Uptake and Distribution of $^{131}I$ by Cultured Mouse Thyroids.[a]

| Medium TSH concentration | Distribution of $^{131}I$ in hydrolysates of mouse thyroid lobes (% Total $^{131}I$) | | | | | Percent $^{131}I$ uptake by mouse lobes |
|---|---|---|---|---|---|---|
| | MIT and DIT | $I^-$ | $T^4$ | $T^3$ | MIT/ DIT | |
| None | 50.81 | 46.15 | 0.94 | 0.07 | 1.56 | 8.50 |
| 20 munits/ml | 75.53 | 10.60 | 8.37 | 0.81 | 1.00 | 16.64 |

[a] Cultures were for 16 hr at pH 8.0. Medium contained 20% v/v lamb serum.

iodide release also was apparent when we examined the effect of varying concentrations of TSH as depicted in Fig. 2. Iodide release, while clearly more sensitive than iodothyronine release, had a lower magnitude of response over the range of 0.1–2.5 mU TSH/ml.

Table III shows the accumulation of iodide by thyroid lobes under different experimental conditions. Accumulation of iodide was not significantly altered by the addition of TSH to the culture medium but was markedly diminished when $KClO_4$ ($10^{-5}$ mole) was present in the medium. Since iodide transport activity is inhibited in the presence of $KClO_4$ these data suggest the preservation of iodide transport activity in the cultured glands at 24 hr.

*Discussion.* Preliminary experiments with organ cultures in our laboratory have indicated that high oxygen content in the incubator is necessary for optimal functional capacity of the cultured thyroid tissue. The addition of lamb serum to the medium and an alkaline pH favored iodothyronine synthesis. When mice were treated with thyroxine prior to sacrifice, *in vitro* TSH markedly enhanced $^{131}I$ uptake and the incorporation of the isotope into iodotyrosine and iodothyronines. However, alkaline pH had no appreciable effect on TSH stimulation of iodide and hormone release.

Unlike the study of Brown and Munro (9) who found maximal discharge of $^{131}I$ after 9 hr of incubation, our data indicate that active proteolysis, induced by TSH, continues for at least 16 hr in our organ culture system. Presumably, this is a reflection of sustained viability of the thyroid lobes under our experimental conditions. Furthermore, a significant increment in iodide and hormone release was evident with as little as 0.5 mU/ml TSH added to the medium.

*Conclusion.* We have demonstrated a culture system employing thyroid lobes from mice which over a 24 hr period seem capable of maintaining good function with respect to the transport of inorganic iodide, the formation of labeled iodothyronines, a capacity to increase hormone formation under TSH stimulation and the ability to release hormone and iodide in a dose dependent manner at relatively low TSH concentrations. The system lends itself to a variety of studies in which the capacity to form thyroxine and triiodothyronine *in vitro* are of the essence.

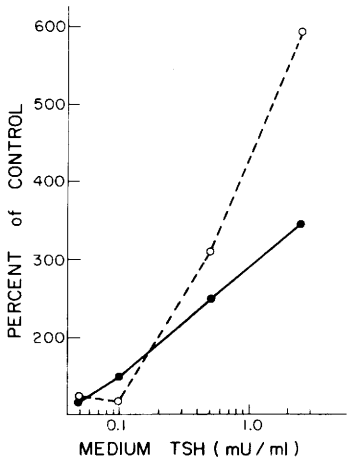


FIG. 2. Effect of TSH concentration on the release of iodide (●) and iodothyronines (○) by cultured mouse thyroid lobes.

TABLE III. Accumulation of Iodide by Thyroid Lobes In Organ Culture.

| Additions to the medium | cpm $^{131}I$/mg of thyroid tissue protein ± SEM |
|---|---|
| none | 52250 ± 5849 |
| TSH (50 munits/ml) | 49800 ±4575 |
| $KClO_4$($10^{-5}$ mole) | 4150 ± 591 |

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