jnm / IN VITRO NUCLEAR MEDICINE

Thyroid Hormones And [^{14}C] Glucose Metabolism in Bacteria

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The effects of triiodothyronine and thyroxine on metabolism and growth of bacteria were studied. It was observed that over a certain range of concentration thyroxine and triiodothyronine produced increase in $^{14}CO_2$ release from [^{14}C]-labeled glucose and also stimulated bacteria growth.

J Nucl Med 18: 736–739, 1977

It has been well established that the thyroid hormones, acting through augmented transcription of DNA, can affect protein synthesis in cell suspensions and tissue cultures in vitro (*1*), but there are very few reports about their effects on bacterial growth and metabolism. Increase in glucose oxidation by yeast cells in the presence of thyroid hormones has been reported (*2,3*). Biswas (*4*) in a brief letter to the editor of *Lancet* has referred to the luxuriant growth of *E. coli* cultures in the presence of thyroxine.

We have studied the effects of thyroxine and triiodothyronine on release of $^{14}CO_2$ as an end-product of bacterial metabolism of [^{14}C]-labeled glucose. During the course of this work, we also observed that these hormones stimulate the growth of microbes.

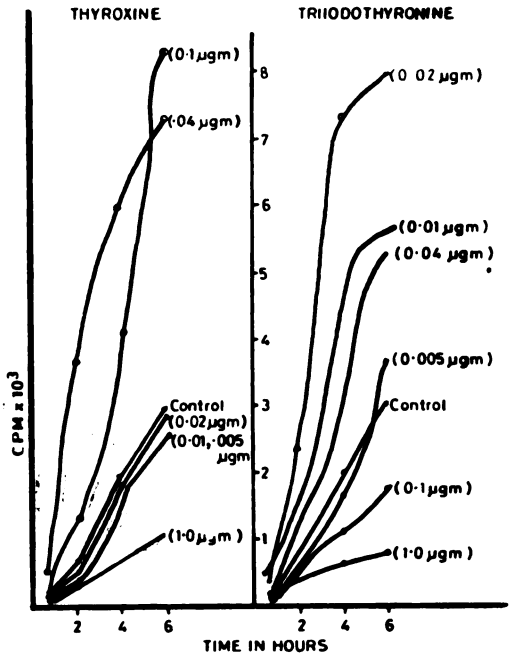

FIG. 1. Effect of varying concentrations (5–1000 ng/ml) of thyroxine and triiodothyronine on release of $^{14}CO_2$ from a culture of *S. aureus* containing [^{14}C] glucose.

MATERIALS AND METHODS

Method for detection of $^{14}CO_2$. The detecting device consists of a liquid-scintillation vial lined with Whatman No. 42 filter paper. The paper has previously been soaked in scintillation fluid (PPO 10 g, POPOP 0.125 g, dioxane 100 ml) and in NaOH and then dried before installation in the vials. Each vial contains within it another narrow cylindrical vial with several holes at the upper end. The inner tube contains nutrient broth, [U-^{14}C] glucose, and a *Staphylococcus aureus* culture. The vials are incubated in a water bath at 37°C, shaken continuously to encourage escape of $^{14}CO_2$ from the aqueous phase. The $^{14}CO_2$ evolved in the inner vial as a result of bacterial metabolism is gradually trapped on the alkali-impregnated filter paper lining the outer vial.

Received Nov. 23, 1976; revision accepted Feb. 1, 1977.

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TABLE 1. EFFECT OF THYROXINE ON $^{14}CO_2$ RELEASE FROM *S. AUREUS* IN SEVEN EXPERIMENTS

Conc. of thyroxine (μg)	$^{14}CO_2$ cpm at 3 hr						
0	9	13	323	3884	6926	6224	2725
0.04	79	94	1274	24791	54141	54076	13501
0.1	138	324	1704	24354	34767	34332	14672
1.0	89	165	593	3632	4638	3809	1377
2.0	23	30	263	641	1411	777	741

TABLE 2. EFFECTIVE DOSE OF THYROXINE FOR VARIOUS ORGANISMS

Organism	Thyroxine μg/ml	Cpm with thyroxine / cpm in control
S. aureus	0.04	6, 8, 8, 8, 7, 7, 9
E. coli	0.1	16
S. typhi	0.1	6
S. dysenteriae	0.1	39
C. welchii	0.1	10
P. vulgaris	1.0	6
M. tuberculosis	10.0	10

The counting was done in a liquid scintillation counter. Since the vials were not opened throughout the incubation period, it was possible to obtain cumulative measurements of the evolved $^{14}CO_2$ by periodic counting. A similar system was described originally by Buddemeyer (*5*), but we have modified his technique to some extent to suit our needs.

Effect of triiodothyronine and thyroxine on $^{14}CO_2$ release. Experiments were performed with concentrations of L-thyroxine ranging from 5–1000 ng/ml. The inner vial held 1–2 ml nutrient broth containing 2 μCi [U-^{14}C] glucose and 0.1 ml of an innoculum containing 10 million cells from an overnight culture of *S. aureus*. Vials were kept in the shaking water bath at 37°C, and the $^{14}CO_2$ was counted in liquid scintillation counter at various time intervals. Similar experiments were carried out with triiodothyronine in concentrations ranging from 5–1000 ng/ml. In similar experiments we also studied the effects of various concentrations of thyroxine on CO_2 production from glucose in other organisms, e.g., *E. coli, S. typhi, C. welchii, S. dysenteriae, P. vulgaris*, and *M. tuberculosis*.

Effect of triiodothyronine and thyroxine on bacterial growth. The control culture and the culture containing thyroxine were plated on nutrient agar plates after $^{14}CO_2$ counting as described above continued up to 4 hr. The plates were then incubated at 37°C for 24 hr.

Incorporation of tritiated thymidine in the presence of triiodothyronine and thyroxine. A *S. aureus* culture (10 million cells in 2 ml nutrient broth) was incubated at 37°C for 24 hr with 10 μCi of [^{3}H] thymidine (sp. act. 10,400 mCi/m *M*) with and without thyroid hormones. At 24 hr the cultures were centrifuged and the cells washed three times with normal saline and then digested for 15 hr in 1 ml hyamine hydroxide. One-tenth ml of hyamine was counted in 15 ml scintillation fluid (*6,7*).

RESULTS

The effects of thyroxine and triiodothyronine on release of $^{14}CO_2$ are shown in Fig. 1. Thyroxine at 5–20 ng/ml had no effect. With increased concentration to 40–100 ng/ml, there was an eightfold increase in counts within 1 hr, but 1.0 μg/ml of thyroxine actually decreased $^{14}CO_2$ release. What was most striking was the very narrow range for the effective concentration of thyroxine. Triiodothyronine produced the same effects, but somewhat earlier and at lower concentrations. The experiments were repeated seven times and same pattern of $^{14}CO_2$ release could be observed every time. The results are given in Table 1.

The experiments were carried out with other organisms (Table 2) and showed that the concentration of thyroxine causing maximum stimulation of $^{14}CO_2$ release varied from 40–1000 ng/ml, but the pattern of $^{14}CO_2$ release was almost the same with each type of organism.

Besides the $^{14}CO_2$ release, it was observed that growth in the culture containing thyroxine was far more abundant than the control cultures, and the data indicating growth paralleled those measuring $^{14}CO_2$ evolution. Up to a point growth was stimulated by increasing amounts of thyroxine, but higher thyroxine concentrations inhibited the growth (Fig. 2).

Table 3 shows that the growth of *S. aureus*, as measured by incorporated tritiated thymidine, is influenced by thyroid hormone in a similar manner.

Bacterial production of $^{14}CO_2$ from labeled glucose has been used to monitor bacterial contamination in various pharmaceutical products and in blood

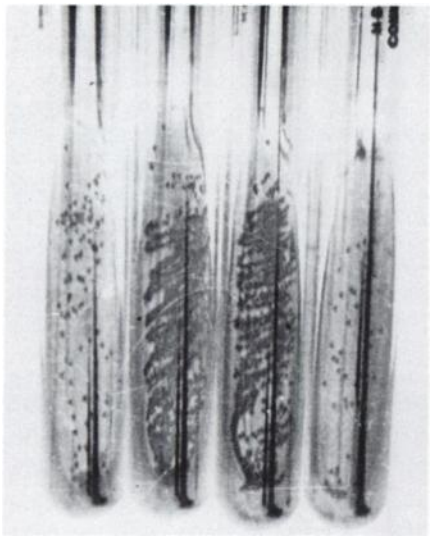

FIG. 2. Effect of thyroxine on growth of a *S. aureus* culture. From left, first nutrient-agar slant is plated from a control culture without thyroxine, second and third from cultures containing 40 and 100 ng thyroxine, respectively, the last plated from a 1-μg/ml culture. Experiments performed with triiodothyronine gave similar results.

cultures (*8*). This in vitro radiorespirometric method reduces time for a sterility test to 8–10 hr, compared with the 8–10 days required by the method in the pharmacopeia. The technique is applied to antibiotic sensitivity testing, where the results can be obtained within 2 hr compared to 24 hr by standard methods (*9*). The addition of thyroxine or triiodothyronine to the culture medium is likely to reduce the $^{14}CO_2$ detection time still further in the preceding methods, since $^{14}CO_2$ is released much earlier from cultures containing triiodothyronine or thyroxine. Results of experiments carried out to show this showed that a *S. aureus* culture containing 60 organisms and 100 ng thyroxine gave 7 times the count rate of the thyroxine-free control, and this reduced the detection period from 4 hr to 0.5 hr (Table 4).

TABLE 3. EFFECT OF THYROID HORMONES ON [^{3}H] THYMIDINE INCORPORATION IN BACTERIA IN TWO EXPERIMENTS

Concentration of T_4 (μg/ml)	Counts/min		Conc. of T_3 (μg/ml)	Counts/min	
0	3219	5716	0	3219	5716
			0.02	12593	14679
0.04	36101	39162	0.04	9171	10013
0.1	7304	7632	0.1	4300	5789
1	3579	4081	1.0	4630	5761

TABLE 4. RELEASE OF $^{14}CO_2$ FROM A SMALL NUMBER OF ORGANISMS (*S. AUREUS*) IN PRESENCE OF THYROXINE IN TWO EXPERIMENTS

No. of organisms	Condition	Counts/min 0.5 hr	2 hr	4 hr	24 hr
60	Without thyroxine	11	13	70	860
		21	40	160	2150
	With thyroxine	79	94	185	2930
		88	124	384	8185

DISCUSSION

We have observed that both triiodothyronine and thyroxine have a stimulatory effect on $^{14}CO_2$ release as well as growth of bacteria over a certain range of concentrations, while above that range it has an inhibitory effect. The optimum concentration varies with the type of bacterium. As in other in vitro systems, we could also observe that triiodothyronine acts much earlier and at lower concentration. It was surprising to observe that the relative effectiveness of thyroxine compared with triiodothyronine was of the same order of magnitude in microbes and in man.

Iodide in the same concentration as thyroid hormones had no effect on growth of organisms. The culture medium used in our experiments contained 50–80 μgm/ml of iodide, and the addition of a fraction of a microgram was highly unlikely to produce any significant difference.

It would be interesting to study the fate of radioactive triiodothyronine or thyroxine in the metabolism of bacteria. Experiments of such a type are under study.

Immediate practical application of these observations would be in augmenting the sensitivity of in vitro radiorespirometric studies for detection of contamination with microorganisms in pharmaceutical and hospital laboratories.

The growth-promoting properties of thyroid hormones may also be useful in the culture of recalcitrant organisms like *M. tuberculosis* and *M. leprae.*

Probably of more fundamental importance is the ubiquitous requirement of thyroid hormones by living cells and the roles these hormones may have in the control and propagation of pathogens in man.

Lastly, can we speculate that the well-established action of thyroid hormones on mitochondria is in a way representative of its action on bacteria if one believes that the mitochondrion is a symbiont bacterium incorporated in the primitive eukaryotic cell?

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