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A STUDY OF THE RATE OF PROTEIN SYNTHESIS BEFORE AND DURING THE ADMINISTRATION OF L-TRIIODOTHYRONINE TO PATIENTS WITH MYXEDEMA AND HEALTHY VOLUNTEERS USING N-15 GLYCINE

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Research Article

A STUDY OF THE RATE OF PROTEIN SYNTHESIS BEFORE AND DURING THE ADMINISTRATION OF L-TRIIODOTHYRONINE TO PATIENTS WITH MYXEDEMA AND HEALTHY VOLUNTEERS USING N-15 GLYCINE [1]

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The purpose of this paper is to report studies of the protein synthesis rate and the size of the metabolic pool using the amino acid, glycine labeled with isotopic nitrogen N-15. Employing the technique described by San Pietro and Rittenberg (1) studies were made on six patients with primary myxedema, four of them before and during the administration of l-triiodothyronine.[2] Five healthy volunteers, one of whom received l-triiodothyronine, are also included in this report.

The N-15 glycine method used in this study is based upon the assumptions that dietary amino acids interact with tissue constituents at a rapid rate (2) and that a dietary amino acid either is used for protein synthesis or is oxidized and its nitrogen excreted.

Figure 1, modified from San Pietro and Rittenberg, illustrates the technique. The metabolic pool (P) is defined as the mixture of nitrogenous compounds derived from the diet and from the breakdown of tissues. Dietary nitrogen enters this pool where part of it is used for protein synthesis. Another part is converted to urea, mixes with the urea already present and is excreted in the urine. The small amount of non-urea nitrogen can be disregarded. The total urea in the body constitutes the urea pool. For the steady

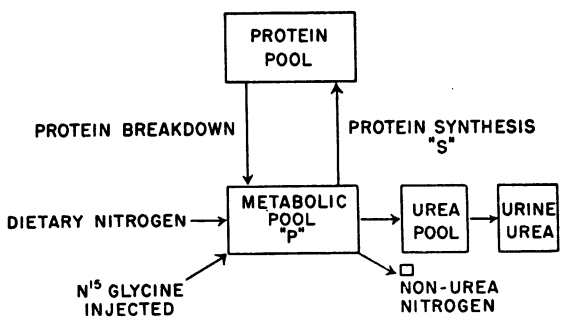

FIG. 1. MODIFIED FROM SAN PIETRO AND RITTENBERG GIVING A SCHEMATIC DIAGRAM OF THE N-15 GLYCINE METHOD

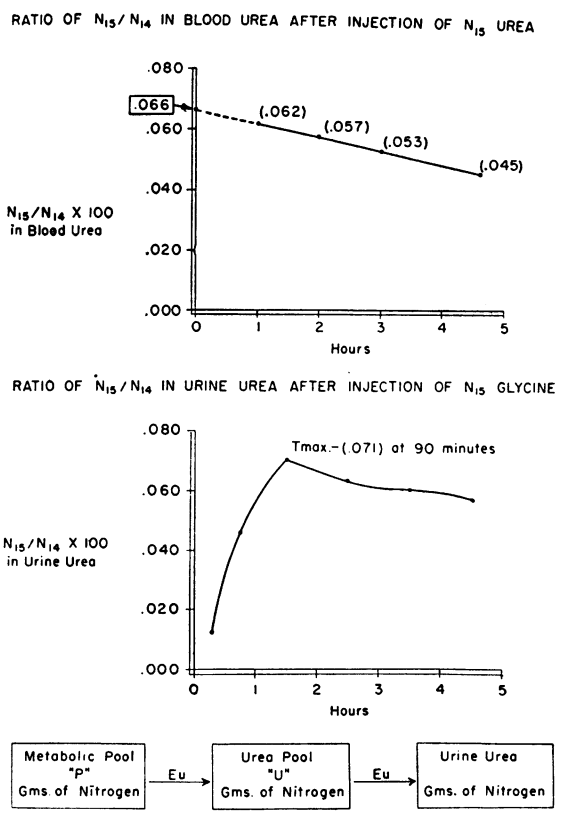

FIG. 2. REPRESENTATIVE CURVES USED TO CALCULATE THE UREA POOL, PROTEIN SYNTHESIS RATE, AND METABOLIC POOL (SEE TEXT)

[1] This study was supported by a grant from the U. S. Public Health Service (C-1815). A preliminary report of this study was given at the International Symposium on Growth Hormone at Henry Ford Hospital, Detroit, Michigan in October, 1954.

[2] Kindly furnished by Dr. Arthur Heming of Smith, Kline, and French Laboratories, Philadelphia, Penna.

TABLE I

Per cent excess of N^{15} urine urea nitrogen

Patient Myxedema	Therapy	Elapsed time (*minutes*) after injection of N^{15} glycine												
		15	20	30	40	45	60	75	90	105	120	150	180	240
R. P.	None		.006		.018		.034	.043	.054	.058	.061	.070	.063	.063
♂	105 Gamma T3 daily—10 days		.015		.037		.057	.070	.073	.072	.072	.068		
	35 Gamma T3 daily—6 months	.011		.028			.081	.054	.061	.047	.064	.066	.066	.064
	35 Gamma T3 daily—14 months													
	70 Gamma T3 daily—1 month		.021		.041		.061	.066	.067		.067		.065	
E. V.	None		.047					.061		.075	.078		.079	.079
♀	105 Gamma T3 daily—10 days					.138	.121	.114	.116			.114	.111	
	53 Gamma T3 daily—2 months	.035		.094		.141	.144	.140	.130	.134	.132	.131	.130	.122
	53 Gamma T3 daily—7 months													
	70 Gamma T3 daily—1 month		.012		.040		.043	.045	.043		.044		.043	
H. P.	None				.009				.033	.044	.048		.051	.053
♀	105 Gamma T3 daily—10 days		.010		.035		.053	.054	.061	.064	.066		.065	.067
B. W.	None	.020									.079	.059	.074	.073
♀	35 Gamma T3 daily—5 days	.020		.045			.114	.095	.095	.087			.090	.093
J. S.	None				.021		.037		.043	.047	.046		.051	.052
♀														
V. G.	None		.020		.054		.073	.076	.080		.083		.085	.067
♀														
Normal														
G. H.	None		.012			.053			.071		.064			.061
♂														
L. G.	None		.013			.066			.148		.120			.112
♂														
L. T.	None		.003				.034	.039	.041	.039	.039		.040	
♂														
S. A.	None				.038			.054	.053	.052	.053	.054	.050	.044
♂	105 Gamma T3 daily—10 days	.095		.097		.090	.144	.086	.065	.063	.063	.066	.066	.064
S. H.	None		.006		.039		.073	.065	.078	.088	.084		.079	.077
♂														

state, dietary nitrogen equals nitrogen excretion and the rate of protein breakdown equals the rate of protein synthesis.

The small amount of the N-15 labeled amino acid glycine which is introduced into the metabolic pool at time zero will be used for protein synthesis or oxidized to urea and excreted. The relative distribution of the N-15 between the protein and urea pools will be a function of the rate of urea excretion and the rate of protein synthesis. If one excretory pathway, *e.g.*, urea (Figure 2), can be completely defined, then it is possible to calculate the rate of protein synthesis and the size of the metabolic pool. This calculation is based upon the assumption that the total transfer of nitrogen from any pool is proportional to the size of that pool. Since the rates of protein synthesis are different in various organs, it is emphasized that this protein synthesis rate is taken as the algebraic sum of all the synthetic rates for the whole organism.

MATERIALS AND METHODS

All subjects were maintained on a constant diet containing approximately 1 Gm. of protein per kilogram of body weight. The nitrogen balance was estimated by analysis of an aliquot of a 24-hour urine specimen and a six-day stool specimen by the macro-Kjeldahl technique (3). An analysis of an aliquot of the diet was made at least once for each study. The patients were all studied while on a metabolic unit and the healthy volunteers carried on their customary activities but were fed on the metabolic unit.

The total body urea was determined by the method of San Pietro and Rittenberg (1) using urea labeled with isotopic nitrogen N-15 (16.0 per cent atom excess) (Figure 2). Approximately three days after determining the total body urea, the subject, while in the fasting state, was given 400 mg. of N-15 glycine (32.0 per cent atom excess) intravenously at 8:00 a.m. One healthy volunteer (S.H.) was given the glycine orally. The urine was then collected over the next four hours at approximately twenty-minute intervals, and the remainder collected to complete a twenty-four-hour specimen (Table I).

An aliquot of each urine specimen was prepared for the mass spectrometer by the method described by

TABLE II

Daily nitrogen balance—GMS

Patient	Before therapy	During therapy	During therapy
Myxedema		105 Gamma T3 daily—10 days	35 Gamma T3 daily—6 months
R. P. ♂	+0.6 +2.0 +0.9 +1.2 +0.7 +1.0 +2.2 +1.9 +1.4 +1.8	−1.1 −1.0 −1.2 −2.9 −5.3 −4.5 −5.3 −5.5 −6.9 −6.9	−0.8 −1.0 −0.2 −0.7 −0.6 −0.6 0.0 +0.6
H. P. ♀	+2.2 +1.5 +3.5 +2.1 +1.5 +2.5 +2.3 +3.3 +3.6 +3.6	+3.6 −0.2 −1.4 −0.9 −3.1 −2.5 −2.7 −5.2 −4.4 −3.1	35 Gamma T3 daily—16 months; then 70 Gamma T3 daily—6 weeks −3.0 −1.3 −0.3 −0.4 −0.5 −1.0 −1.1 −0.4
J. S. ♀	+2.0 +2.9 +2.5 +0.6 +2.1 +1.7 +1.0 +1.6 +2.9 +1.6		
B. W. ♀	+0.3 +0.9 −1.3 +1.8 −0.1 −0.2 +0.3	35 Gamma T3 daily—10 days 0.0 +0.5 −0.9 −2.3 +0.2	
E. V. ♀	+2.2 +2.8 +2.5 +2.2 +1.8 +2.3 +2.1 +1.7 +2.1 +2.2	105 Gamma T3 daily—10 days +2.9 +1.3 −0.8 −1.8 −3.1 −3.2 −3.2 −4.2 −2.6	53 Gamma T3 daily—2 months −1.6 −0.7 −1.2 0.0 −0.9 −0.5 −1.5 −1.2
V. G. ♀	+0.2 +0.6 +0.8 +0.6 +1.0 +1.2 +0.8 +0.4 +1.2 +0.2		53 Gamma T3 daily—7 months 70 Gamma T3 daily—1 month −0.2 0.0 −0.1 0.0 −0.7
Normal			
S. H. ♂	−0.1 +0.1 −0.1 −0.3 −0.3 +0.2 +0.6 +1.0 +1.4 +0.9		
L. T. ♂	+1.5 −0.3 −0.1 −0.7 +1.5 −0.6 −0.5 −1.2 −0.3 0.0		
G. H. ♂	+1.1 +1.1 +1.3 +0.3 +1.6 +2.2 +0.9 −0.1 +0.4 +0.1		
L. G. ♂	−3.0 −1.9 −2.4 −1.2 −0.4 −0.5 −0.2 +0.1 −0.6 −0.4		
S. A. ♂	−0.6 +0.4 +0.4 −0.6 +1.2 +0.3 0.0 −1.7 −1.0 −1.1	105 Gamma T3 daily—10 days −1.0 −0.7 −0.7 −1.4 −1.4 −1.2 −0.9 −2.0 −2.0	

Sprinson and Rittenberg (4). The N-15/N-14 ratio was then determined by means of the mass spectrometer (Consolidated Nier type) with a single slit beam with an accuracy of ± .002 per cent excess.

The studies were carried out in the following subjects:

1. Five studies on five healthy volunteers—L. G., G. H., L. T., and S. A., (♂) and one study on S. A. while receiving l-triiodothyronine 100 micrograms daily over a ten-day period.

2. Fourteen studies on six patients with primary myxedema—R. P. (♂), H. P., E. V., B. W., V. G., and J. S. (♀) in positive nitrogen balance (Table II).

3. Patients R. P., E. V., and H. P. were studied while receiving 100 micrograms of l-triiodothyronine daily for ten days for the first time; and patient B. W. while receiving 35 micrograms daily for the first time.

4. Patient R. P. was restudied after having been on 35 micrograms of l-triiodothyronine for six months and again after receiving 35 micrograms daily for sixteen months and 70 micrograms daily for six weeks. Patient E. V. was restudied after receiving 53 micrograms daily for two months, and again after receiving 53 micrograms daily for seven months and 70 micrograms daily for one month.

RESULTS

The protein synthesis rate, expressed as milligrams of nitrogen per Kg. per 24 hours, was decreased in the patients with myxedema as compared with the healthy volunteers. The average rate for the six patients with myxedema was 354 mg. nitrogen per Kg. per 24 hours (Range 180 to 470 mg. nitrogen) as compared to 756 mg. nitrogen per Kg. per 24 hours (Range 610 to 970 mg. nitrogen) for the healthy volunteers (Table III). The pool size (P) was not consistently altered.

Five of the patients with primary myxedema were in slight to moderate positive nitrogen balance as determined by classical nitrogen balance technique (Table II). The administration of l-triiodothyronine in daily doses of 105 micrograms to patients R. P., E. V., and H. P. resulted in markedly negative nitrogen balance. The protein synthesis rate in patient E. V. measured during this period did not change, while that of H. P. also measured during this period tended to decrease, 490 mg. nitrogen to 318 mg. of nitrogen per Kg. per 24 hours. Patient B. W. received only 35 micrograms of l-triiodothyronine daily for six days and there was no change in nitrogen balance or in the protein synthesis rate during this time.

Patient E. V. was restudied after having re-

TABLE III

Patient	Wt.	Therapy	S	P	U
Myxedema	Kg.	T3	Mg. / Kg. / 24 Hrs.	Mg. / Kg.	Gms.
R. P. ♂	85.2		180	7.0	8.3
	80.1	105 Gamma Daily - 10 Days	370	9.0	7.9
	83.0	35 Gamma Daily - 6 Months	370	4.0	6.6
	80.0	35 Gamma Daily - 14 Months			
		70 Gamma Daily - 1 Month	412	6.5	6.2
E. V. ♀	70.0		350	17.4	5.6
	66.5	105 Gamma Daily - 10 Days	328	4.2	5.0
	66.0	53 Gamma Daily - 2 Months	482	7.0	3.9
	73.0	53 Gamma Daily - 7 Months			
		70 Gamma Daily - 1 Month	1300	22.4	3.9
H. P. ♀	77.0	None	490	21.0	6.1
	74.0	105 Gamma Daily - 10 Days	318	10.0	7.6
B. W. ♀	60.5	None	403	8.6	5.6
	60.0	35 Gamma Daily - 6 Days	400	4.5	5.5
J. S ♀	62.5	None	470	19.0	7.0
V. G. ♀	66.8	None	233	11.5	6.1
Normal					
S. H. ♂	76		670	15.0	4.1
L. T. ♂	81		970	17.0	5.7
G. H. ♂	77.8		850	13.0	5.0
L. G. ♂	77.3		610	12.0	5.0
S. A. ♂	71.0		680	10.0	5.4
	72.0	105 Gamma Daily - 10 Days	126	2.5	6.5

* T-3-L-triiodothyronine, S-protein synthesis rate, P-metabolic pool, U-urea pool.

ceived 53 micrograms of triiodothyronine daily for two months. There was a small increase in synthesis rate, 328 to 482 mg. nitrogen per Kg. per 24 hours. She was still in minimal negative nitrogen balance at this time. After receiving 50 micrograms daily for seven months and 70 micrograms daily for one month the synthesis rate had increased to 1300 mg. nitrogen per Kg. per 24 hours. At this time she was in nitrogen equilibrium.

Patient R. P. was restudied after receiving 35 micrograms of l-triiodothyronine daily for six months. The synthesis rate had increased from 180 to 370 mg. nitrogen per Kg. per 24 hours. After receiving 35 micrograms daily for sixteen months and 70 micrograms daily for six weeks restudy showed a synthesis rate of 412 mg. nitrogen per Kg. per 24 hours. He was in nitrogen equilibrium on the day the last study was carried out.

The administration of 105 micrograms of l-triiodothyronine daily for ten days to a healthy volunteer (S. A.) caused no definite decrease in weight and very minimal negative nitrogen balance but decreased the synthesis rate from a pretreatment level of 680 to 126 mg. nitrogen per Kg. per 24 hours.

DISCUSSION

The protein synthesis rate expressed as milligrams of nitrogen per kilogram per twenty-four hours was consistently decreased in the six patients with primary myxedema (Figure 3, Table III) as compared to the healthy volunteers. This occurred despite the fact that the patients with myxedema were in moderately positive nitrogen balance by classical nitrogen balance studies.

During the first few days that l-triiodothyronine was first administered there was no consistent change in the protein synthesis rate (Table III). In three patients there was little or no change while in one there was an increase toward the normal. The results obtained during this period may not be valid because the steady state has been disturbed and a new one not yet established as manifested by the increasing negative nitrogen balance (Table II).

The continued administration of l-triiodothyronine to two myxedematous patients with the attainment of the euthyroid state and a new steady state as shown by nitrogen equilibrium resulted in a return to a normal protein synthesis rate in one and a marked increase with a return toward normal in the other (Figure 4).

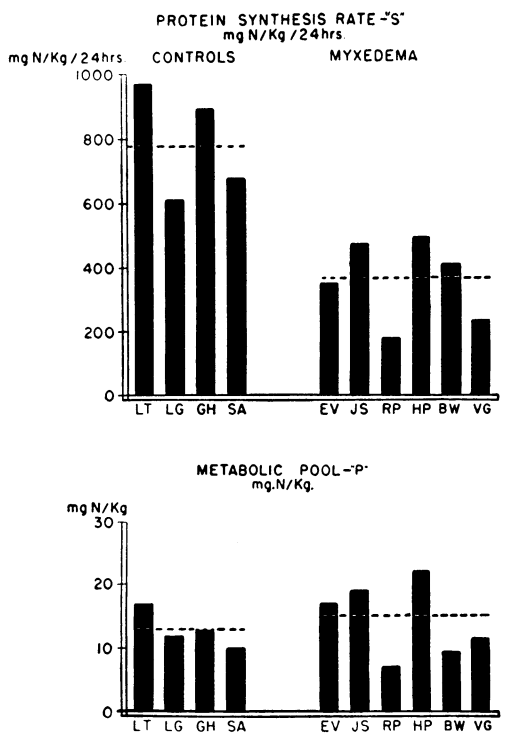

FIG. 3. A COMPARISON OF THE RATE OF PROTEIN SYNTHESIS AND THE SIZE OF THE METABOLIC POOL IN HEALTHY VOLUNTEERS AND PATIENTS WITH MYXEDEMA

The broken horizontal lines represent the average for the various groups.

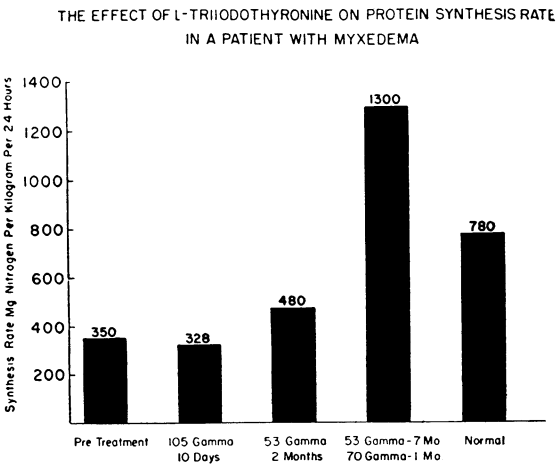

FIG. 4. A COMPARISON OF THE PROTEIN SYNTHESIS RATE OF PATIENT (E. V.), BEFORE AND AFTER THERAPY WITH L-TRIIODOTHYRONINE, WITH THE HEALTHY VOLUNTEERS

DuToit (5) has obtained results *in vitro* similar to these reported here by studying the effect of thyroid hormone on the incorporation of the amino acid alanine into the proteins of rat liver slices using alanine tagged with radioactive carbon, C-14. Thyroidectomy markedly reduces the incorporation rate and the administration of thyroxine to the athyreotic animal restores the incorporation rate to normal. He reports no studies of the effect of thyroxine on normal animals.

From the above animal studies and the results we have obtained in patients with myxedema it would appear that the absence of the thyroid hormone results in an overall decrease in protein synthesis which is corrected by the administration of thyroid hormone. By the present technique we are not able to calculate the rate of breakdown of tissue protein. Olesen, Heilskov, and Schønheyder (6) have recently introduced a new mathematical interpretation of data of this kind. By extending the period of observation over 360 hours rather than 24 hours it is possible to calculate, in addition to pool size and protein synthesis rate, the rate of protein breakdown. Data are being collected according to this technique in patients with myxedema as it seems important to try to delineate what is happening in the relatively acute stage when the patient is in profound negative ni-

trogen balance yet the protein synthesis rate showed little change (Table II, Figure 4). This could mean that "myxedematous tissue" is being catabolized and excreted and that there is a lag period before new normal tissue is formed. The present data are only suggestive and need confirmation by the technique of Olesen, Heilskov, and Schønheyder (6).

The profound negative nitrogen balance and decrease in weight (Figure 5) produced by l-triiodothyronine in the patients with myxedema have been previously reported (7). A similar effect has been reported using dessicated thyroid or thyroxin (8, 9). The administration of l-triiodothyronine in a daily dose of 105 micrograms for ten days to a healthy volunteer (S.A.) produced very minimal negative nitrogen balance and no weight loss. However, there was a marked decrease in the protein synthesis rate from 680 mg. nitrogen per Kg. per 24 hours before therapy to 126 mg. nitrogen per Kg. per 24 hours while receiving the drug. From this single study it would appear that an excess of thyroid hormone also decreases the rate of protein synthesis. We are now studying patients with hyperthyroidism to see if they demonstrate this abnormality.

Rupp, Paschkis, and Cantarow (10) have shown that in the thyroidectomized rat physiological doses of thyroxine decrease nitrogen excretion which they interpret as a protein anabolic effect. Large amounts of thyroxine given to the intact rat caused increased nitrogen excretion or a protein catabolic effect. Our results in man presented here are consistent with the above studies in the rat.

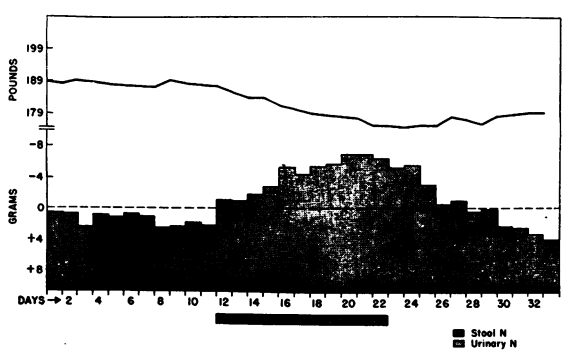

FIG. 5. NITROGEN BALANCE STUDY ON PATIENT R. P.

Results plotted so that below the dotted line is positive nitrogen balance and above the dotted line is negative nitrogen balance.

CONCLUSIONS

1. The protein synthesis rate as measured by the N-15 glycine technique was decreased in six patients with primary myxedema.

2. The administration of l-triiodothyronine restored the protein synthesis rate to a normal level.

3. The administration of triiodothyronine to a healthy volunteer also decreased the rate of protein synthesis suggesting that an excess of thyroid hormone will also decrease the rate of protein synthesis.

4. This N-15 glycine technique appears to offer insight into protein metabolism in man which is not obtainable by classical nitrogen balance studies.

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