

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 GLYCINE¹

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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

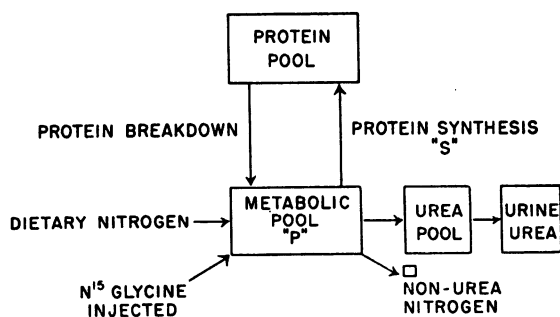
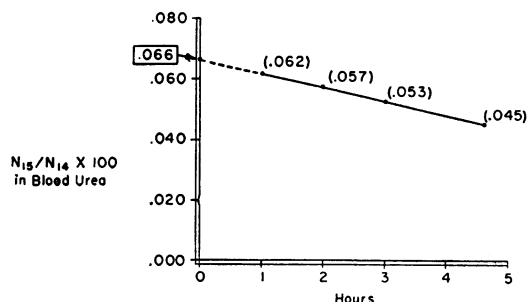


FIG. 1. MODIFIED FROM SAN PIETRO AND RITTENBERG GIVING A SCHEMATIC DIAGRAM OF THE N-15 GLYCINE METHOD

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RATIO OF N_{15}/N_{14} IN BLOOD UREA AFTER INJECTION OF N_{15} UREA



RATIO OF N_{15}/N_{14} IN URINE UREA AFTER INJECTION OF N_{15} GLYCINE

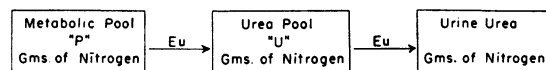
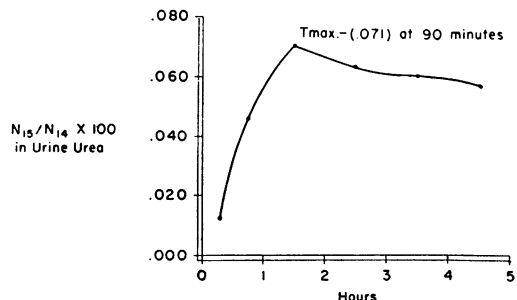


FIG. 2. REPRESENTATIVE CURVES USED TO CALCULATE THE UREA POOL, PROTEIN SYNTHESIS RATE, AND METABOLIC POOL (SEE TEXT)

TABLE I
Per cent excess of N¹⁵ urine urea nitrogen

Patient Myxedema	Therapy	Elapsed time (<i>minutes</i>) 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
	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
S. H. ♂	105 Gamma T3 daily—10 days	.095		.097		.090	.144	.086	.065	.063	.063	.066	.066	.064
	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 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 re-study 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 pre-treatment 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

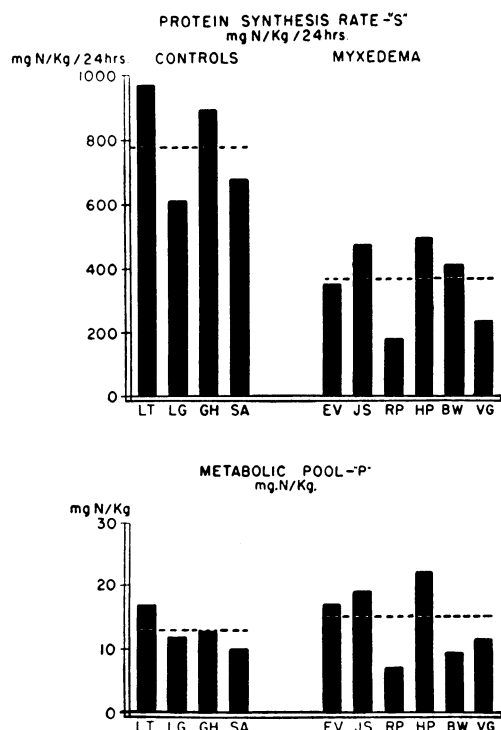


FIG. 3. A COMPARISON OF THE RATE OF PROTEIN SYNTHESIS AND THE SIZE OF THE METABOLIC POOL IN HEALTHY VOLUNTEERS AND PATIENTS WITH MYXEDEMA

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

THE EFFECT OF L-TRIIODOTHYRONINE ON PROTEIN SYNTHESIS RATE IN A PATIENT WITH MYXEDEMA

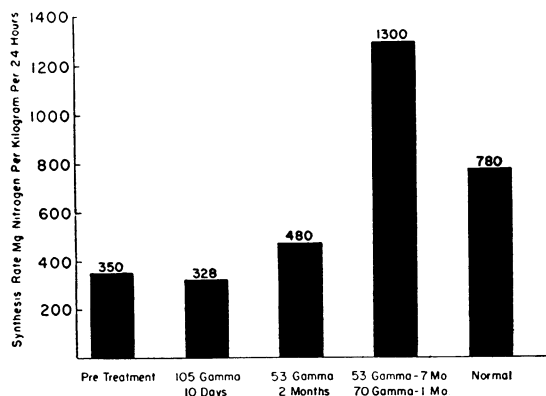


FIG. 4. A COMPARISON OF THE PROTEIN SYNTHESIS RATE OF PATIENT (E. V.), BEFORE AND AFTER THERAPY WITH L-TRIIODOTHYRONINE, WITH THE HEALTHY VOLUNTEERS

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).

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 thyroxine (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.

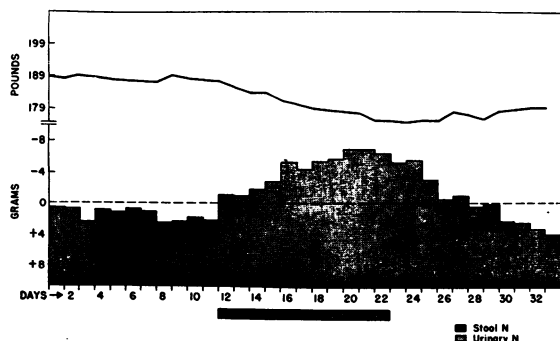


FIG. 5. NITROGEN BALANCE STUDY ON PATIENT R. P.

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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