Conversion of Thyroxine to Triiodothyronine in Normal Human Subjects

Abstract. *The conversion of thyroxine to triiodothyronine, previously demonstrated in athyreotic human subjects, has been investigated in normal subjects who were given intravenous injections of purified thyroxine labeled with carbon-14 in ring A and in the alanine side chain. Evidence for the conversion of T4 to T3 was provided by the finding of carbon-14 in the T3 fraction isolated from serums. It is estimated that an appreciable fraction of T4 may be transformed to T3 in normal man.*

Thyroxine (T4) is converted to triiodothyronine (T3) in athyreotic or hypothyroid human subjects maintained on synthetic sodium L-thyroxine administered orally (*1*).

The finding of T3 in the serum was verified by studies in which the oral dose of T4 was supplemented with [^{125}I]T4 for a week or more. In addition to radioactive T3, labeled tetraiodothyroacetic acid was also identified in the serum.

The conversion of T4 to T3 was not peculiar to the oral route of administration but was indeed a function of extrathyroidal metabolism since the same results were observed after intravenous administration.

Considerable evidence tended to rule out the possibility that the T3 in the serum arose as the result of artefactual deiodination of T4, either during storage of serums or during separative or analytic procedures. Purified [^{125}I]T4 with negligible T3 contamination remained similarly free of T3 during storage, as judged by repeated chromatographic analyses at intervals during a 3-week period. The addition of high concentrations of stable T4 to serum, which was then carried through the entire analytic procedure, resulted in no detectable increase from the previously measured T3 concentration.

This demonstration of conversion of T4 to T3 in athyreotic subjects raised the question of the extent of conversion of T4 to T3 in normal subjects.

In our work, T4 labeled with ^{14}C in ring A and in the alanine side chain was used. ^{14}C-Labeling was used to eliminate any possible iodination as the source of triiodothyronine radioactivity, as well as any possibility of exchange of a radioactive iodine label with iodine of T3 already present.

The five subjects were healthy volunteers devoid of significant disease, including thyroid disease. Tests were made of thyroidal uptake of tracer ^{131}I, serum thyroxine concentration, serum protein bound iodine, total serum protein, binding capacity of thyroxine binding globulin, and iodine excretion in a 24-hour sample of urine (*2*). The volunteers received intravenous injections of purified [^{14}C]T4 as 8.1 μg (1.4 μc) of T4 daily for ten consecutive days. The concentrations of T4 in the serum were measured at the Boston Medical Laboratory by the displacement method. The T3 concentrations were measured in our laboratory by a separation and displacement procedure (*3*). The serum hormones obtained from the resin columns were subjected to descending paper chromatography with an improved solvent system (hexane, teritary amyl alcohol, ammonia), which is effective for maximum separation between T4 and T3 peaks (*3*). Scanning of chromatograms from consecutive serums showed progressively greater definition of T3 and tetraiodothyroacetic acid peaks from the base line, attributed to the rising specific activity of the circulating T4. Serums from the subjects showed approximately constant values for total T4 and T3 concentrations throughout the studies, an indication of a metabolic steady state. The initial and final concentrations of T4 in the serums agreed within 10 percent. Concentrations of T3 in all serums had a coefficient of variance of 15 percent or less.

On descending chromatography, the serum extracts revealed a ^{14}C-labeled compound having the chromatographic mobility of T3. A typical scan of the radioactivity of a descending chromatogram of the ^{14}C compounds in a serum (subject 2) 5 days after the series of ten daily intravenous injections (Fig. 1) shows a moderate peak at the origin, a very large T4 peak and small but distinct peaks in the areas occupied by tetraiodothyroacetic acid and by T3.

Purified [^{3}H]T4 had been added to the serum as a control before any procedures. For quantitative determination of the percentage of [^{14}C]T3, the ^{14}C in T3 areas was determined after the sample was twice subjected to one-dimensional descending paper chromatography. For ready identification of the T3 area, [^{131}I]T3 was also added to serums prior to the initial chromatography of samples from subjects 3–5. After being subjected to descending paper chromatography, the ^{131}I peak of added T3 was quite easily seen on scanning. The material of the T3 zone was eluted, the chromatography was repeated, and the labeled T3 was again eluted. The added ^{131}I was assayed in a scintillation well counter and the result was used as a recovery standard for T3 in the serum. After the two chromatographic separations recovery was approximately 20 to 30 percent of the added [^{131}I]T3. An interval of more than 12 weeks was allowed for decay of ^{131}I radioactivity to background levels, as verified by well counter. Alternatively, appropriate subtractions were made for residual ^{131}I in the ^{14}C channel.

Thus with added [^{131}I]T3, in subject 3 there was no detectable ^{3}H in the T3 zones of 8 of 11 chromatographic strips. The absence of detectable ^{3}H radioactivity signified that, in these strips, no contaminating [^{3}H]T4 had

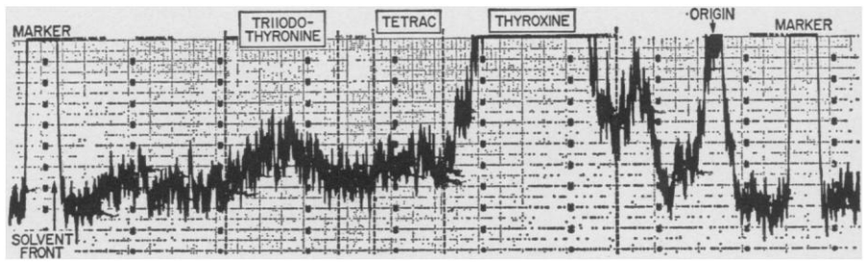

Fig. 1. Scan of radioactivity in paper chromatogram of serum of subject 2, 5 days after the last of ten daily intravenous injections of [^{14}C]T4. In addition to the very large truncated T4 peak and the origin peak, the small T3 and tetraiodothyroacetic acid (Tetrac) peaks are evident. The peak between T4 and the origin is unidentified.

Table 1. Conversion of T4 to T3 in normal human subjects; SA, specific activity.

Day after first injection	Subject 4		Subject 5	
	Serum ^{14}C as T3 (%)	SA T3/T4	Serum ^{14}C as T3 (%)	SA T3/T4
2	0.72	0.25		
3			1.53	0.49
4	1.04	0.36		
5			1.72	0.52
6	1.29	0.44		
7			2.20	0.69
8	1.17	0.40		
9			0.95	0.28
10	1.23	0.42		
11			1.16	0.36
12	1.11	0.39		
13			1.47	0.46
15			1.11	0.36
17			1.69	0.54
19			1.73	0.55
21			1.59	0.49
Mean	1.2*	0.40*	1.5	0.48
S.D.	±0.10*	0.03*	±0.36	±0.12

* From day 4 on.

remained, and there had been no appreciable conversion of T4 to T3 in vitro. From day 3 on, [^{14}C]T3 was present in the serum, and by this time it had reached an apparent plateau. The data were interpreted to support the conversion of administered labeled T4 to T3 in vivo. In this set of serums from subject 3, corrections were required for residual ^{131}I.

The serums of subjects 4 and 5 had no detectable ^{131}I at the time of ^{14}C assay, which showed mean values of 1.2 and 1.5 percent of the total serum radioactivity in the T3 zones (Table 1).

Again, the principal inference from the data obtained was the conversion of T4 to T3 in normal human volunteers, a result in agreement with the demonstrated conversion of T4 to T3 in athyreotic human subjects maintained on T4 (*1*).

The percentages of [^{14}C]T3 in the serums of subjects 4 and 5 were 1.2 and 1.5 percent. Since T3 has a much more rapid fractional turnover than T4, even as little as 1.5 percent of radioactivity in the serum suggests appreciable conversion. Despite the fact that the normal concentration of T3 in serum is only about 1/30 that of T4 (*3*), the absolute removal rate is almost as great (*3, 4*). Thus the normal rate of disposal of T4 approximates 80 μg/day, while that of T3 is approximately 60 μg/day (*3, 5*). The volume clearance of T4 is about 1 liter/day, whereas T3 has a clearance of approximately 22 liter/day (*5*). On this basis, one may estimate that 1.5 percent of serum radioactivity as T3 could signify 0.33 liter/day (0.015 × 22) as the probable fraction converted of the daily T4 clearance of 1 liter. This would then suggest that as much as one-third of the T4 disposal could occur by conversion to the rapidly metabolized T3 in normal humans. Obviously further kinetic data would be needed to verify the extent of this route of transformation.

The converse problem is the relative contribution of T3 formed by conversion from T4 to the circulating T3 in comparison with that secreted by the thyroid gland. If all T3 were made from T4, the ratio of the specific activity of T3 to that of T4 after equilibration would be 1.00. If half the T3 were from T4, the ratio would be 0.5. In subjects 4 and 5 (Table 1) the ratios were 0.40 and 0.48, signifying that more than one-third of T3 may arise from conversion in normal human subjects.

Inasmuch as T3 is three to four times as potent as T4 and has recently been considered to produce the major thyroid hormonal effect (*3*) our findings raise the question whether T4 itself has any primary action or exerts its effect only after transformation to T3.

KENNETH STERLING
MILTON A. BRENNER
EDWARD S. NEWMAN
Protein Research Laboratory and *Veterans Administration Hospital, Bronx, New York 10468*, and *Department of Pathology, Columbia University College of Physicians and Surgeons, New York 10032*

References and Notes

1. L. E. Braverman, S. H. Ingbar, K. Sterling, *J. Clin. Invest.* **49**, 855 (1970).
2. Clinical work-up, injections, and collection of serums were carried out by Dr. C. S. Pittman.
3. K. Sterling, D. Bellabarba, E. S. Newman, M. A. Brenner, *J. Clin. Invest.* **48**, 1150 (1969).
4. K. Sterling, J. C. Lashof, E. B. Man, *ibid.* **33**, 1031 (1954); K. Sterling and R. B. Chodos, *ibid.* **35**, 806 (1956); S. H. Ingbar and N. Freinkel, *ibid.* **34**, 808 (1955); D. A. Fisher and T. H. Oddie, *J. Clin. Endocrinol.* **24**, 733 (1964).
5. K. A. Woeber, R. J. Sobel, S. H. Ingbar, K. Sterling, *J. Clin. Invest.* **49**, 643 (1970).
6. Supported by grant AM-10739 from the PHS and grant P-461 from the American Cancer Society. We thank Dr. Hiram Hart for help in the mathematical formulation of the kinetics of the conversion of T4 to T3.

18 May 1970

Serotonin-Containing Neurons in Brain: Depression of Firing by Monoamine Oxidase Inhibitors

Abstract. *Monoamine oxidase inhibitors were administered to rats while the activities of single, serotonin-containing neurons of the midbrain raphe nuclei were being monitored with microelectrodes. All the inhibitors tested (pargyline, tranylcypromine, phenelzine, iproniazid) caused depression of raphe unit firing rate. The ability of monoamine oxidase inhibitors to depress raphe units was impaired by prior treatment with* p-*chlorophenylalanine, an inhibitor of serotonin synthesis.*

Inhibition of monoamine oxidase (MAO) markedly increases the concentration of serotonin (5-hydroxytryptamine; 5-HT) in brain (*1*). By the histochemical fluorescence method for monoamines, at least some of this increase has been shown to take place within the 5-HT–containing neurons of the midbrain raphe nuclei (*2*). We now report the monitoring of the activity of single neurons in the raphe nuclei during the administration of MAO inhibitors to determine whether this observed increase in 5-HT content is associated with an alteration in rate of unit firing. We found that MAO inhibitors of various chemical structures produce a marked depression of raphe unit firing; these results demonstrate for the first time that MAO inhibitors modify the physiological activity of monoamine-containing neuronal units in the brain.

Extracellular spikes of single, midbrain raphe neurons were recorded (*3*). In brief, male rats (Charles River, cesarean delivered; 250 to 275 g) were anesthetized with chloral hydrate and mounted in a stereotaxic apparatus. A tungsten microelectrode, with a tip diameter of approximately 1 μm, was lowered through a burr hole into the midbrain raphe area by means of a hydraulic microdrive. Electrode signals were fed into a high impedence amplifier and then displayed on an oscilloscope screen. The firing rate of an individual unit was followed with an electronic counter whose analog output was plotted on a potentiometric re-