nockites or the associated high-grade metamorphic rocks of granulite facies show a close correspondence in age ($\times 10^6$ years) (*14, 24*): Adirondacks region of New York, 1120; eastern India, 1300 to 1520; Podlasie, Poland, 1250 to 1350; and Sweden and Norway, 900 to 1300.

According to the compilation of Engel *et al.* (*25*), the ages of the rocks of the granulite facies show a significant modal class around $1200:10^6$ years ago. It seems that during this period in the earth's history charnockites must also have recrystallized, and temperatures in the range of 800° to 900°C were attained at shallow depths of 6 to 12 km. Such geothermal gradients of the order of 70° to 100°C per kilometer would also lead to the melting of the mantle rocks. The significance of the information on the pressure and temperature of formation of charnockites presented above cannot be fully realized unless age data on charnockites are collected for the different regions of the world. The age relationships could show whether the formation of charnockites was due to a global thermal event such as that proposed by Herz (*26*) for anorthosite formation or whether they formed in isolated events unrelated in space and time.

S. K. SAXENA

Department of Geology, Brooklyn College, City University of New York, Brooklyn 11210

References and Notes

1. R. A. Howie, *Sci. Progr. (London)* **52**, 628 (1964).
2. P. M. Hurley and J. R. Rand, *Science* **164**, 1229 (1969).
3. C. Muthuswami, *Proc. Indian Acad. Sci.* **37**, 730 (1956).
4. R. A. Howie, *Trans. R. Soc. Edinburgh* **62**, 725 (1955).
5. F. H. Hubbard, *Geol. Foeren. Stockholm Foerh.* **97**, 223 (1976).
6. "Charnockitization" refers to reactions leading to the metamorphism of igneous or sedimentary rocks to charnockites.
7. These charnockites were emplaced during the time of granulite facies metamorphism and must have recrystallized as indicated by pyroxene geothermometry (see text).
8. R. Kretz, *J. Geol.* **67**, 371 (1959).
9. S. K. Saxena, *Thermodynamics of Rock-Forming Crystalline Solutions* (Springer-Verlag, New York, 1973), p. 180.
10. C. Leelanandam, *Mineral. Mag.* **36**, 153 (1967); S. K. Saxena, *Contrib. Mineral. Petrol.* **20**, 177 (1969); ——— and N. B. Hollander, *Neues Jahrb. Mineral. Monatsh.* **2**, 85 (1969); S. K. Saxena, unpublished data.
11. S. K. Saxena, *Am. Mineral.* **53**, 1674 (1968).
12. I. S. McCallum, thesis, University of Chicago (1968).
13. F. R. Boyd, *Geochim. Cosmochim. Acta* **37**, 2533 (1973); J. Mercier and N. Carter, *J. Geophys. Res.* **80**, 3349 (1975).
14. A. E. J. Engel, C. G. Engel, R. G. Havens, *J. Geol.* **72**, 131 (1964).
15. M. Ross and J. S. Huebner, paper presented at the International Symposium on Geothermometry and Geobarometry, Pennsylvania State University, October 1976.
16. S. K. Saxena, *Am. Mineral.* **61**, 643 (1976).
17. J. Gross, in preparation.
18. S. A. Morse, *J. Petrol.* **11**, 221 (1970); *Carnegie Inst. Washington Yearb.* **67**, 120 (1968).
19. F. R. Boyd, in *Researches in Geochemistry*, P. H. Abelson, Ed. (Wiley, New York, 1959), pp. 377–397.
20. R. F. Mueller and S. K. Saxena, *Chemical Petrology* (Springer-Verlag, New York, 1977), p. 177.
21. W. C. Luth, *J. Petrol.* **8**, 372 (1967).
22. W. Ryka, *Int. Geol. 23rd Congr. Abstr.* (1968).
23. V. Rama Murthy, personal communication.
24. V. Aswathanarayana, *Can. J. Earth Sci.* **5**, 59 (1968); E. Welin, *Geol. Foeren. Stockholm Foerh.* **88**, 29 (1966); P. Pasteels and J. Michot, *Nor. Geol. Tidsskr.* **55**, 111 (1975).
25. A. E. Engel, S. P. Itson, C. G. Engel, D. M. Stickney, E. J. Cray, Jr., *Geol. Soc. Am. Bull.* **85**, 843 (1974).
26. N. Herz, *Science* **164**, 944 (1969).

14 April 1977; revised 9 June 1977

Pituitary Nuclear 3,5,3′-Triiodothyronine and Thyrotropin Secretion: An Explanation for the Effect of Thyroxine

Abstract. *An excellent correlation was observed between nuclear triiodothyronine (T_3) and the ensuing suppression of thyrotropin (TSH) after a single intravenous injection of T_3 to thyroidectomized (hypothyroid) rats. At 1 and 2 hours after injection of thyroxine (T_4), in amounts equally potent to the administered T_3 in terms of acute suppression of TSH, the same quantities of T_3 were found in the pituitary nuclei. Virtually no nuclear T_4 was present, and plasma T_3 was negligible at these short intervals after T_4 injection. These results suggest that suppression of TSH release in hypothyroid rats occurs by interaction of T_3 with the nuclear receptor of the thyrotroph. After T_4 injection, the T_3 found in the nucleus is derived from rapid intrapituitary monodeiodination.*

There is strong evidence supporting the cell nucleus as the site of initiation of thyroid hormone action (*1*). The degree of occupancy of nuclear binding sites by triiodothyronine (T_3) has been correlated with the quantity of malic enzyme and mitochondrial α-glycerophosphate dehydrogenase in hepatic tissue (*1, 2*) and with growth hormone synthesis and inhibition of prolactin secretion in GH_1 pituitary cells in culture (*3*). Physiological studies have indicated that in the liver, thyroxine (T_4) produces its thyromimetic effect primarily by serving as a source of T_3. Inhibition of the conversion of T_4 to T_3 by 6-*n*-propyl-2-thiouracil (PTU) blocks a significant fraction of the hepatic effect of T_4 (*4*).

Recently, Larsen and Frumess (*5*) reported comparative studies on the effects of T_3 and T_4 in thyroidectomized rats. In these experiments, T_4 (800 ng per 100 g body weight) caused the same degree of acute inhibition of thyrotropin (TSH) release as rapidly as did T_3 at 70 ng per 100 g. Interestingly, the serum T_3 concentrations in animals injected with T_4 increased only minimally into the low to normal range, contrasting with the sharp elevation in the animals injected with T_3. In the same experiments, PTU pretreatment did not block the TSH-suppressive effect of T_4. From previous studies with hepatic nuclear T_3 receptors and those from GH_1 pituitary cells in culture, it would be assumed that T_4 binds to the T_3 pituitary nuclear receptor with at most one-tenth the affinity of T_3 (*1, 6*). Nevertheless, our data, as well as those of other investigators, could be interpreted as showing that T_4 has a direct inhibitory effect on TSH release (*7*). Alternatively, in light of previous studies demonstrating tracer T_3 in the anterior pituitary after injection of tracer T_4 (*8*), we considered the possibility that T_4 may be rapidly monodeiodinated to T_3 in the thyrotroph, providing T_3 directly to the nuclear receptor. The evidence presented in this report indicates that this, in fact, occurs, and that suppression of plasma TSH after either T_3 or T_4 injection is correlated both temporally and quantitatively with the occupancy of the pituitary nuclear receptor sites by T_3.

To establish a correlation between pituitary nuclear T_3 receptor occupancy and serum TSH, we used the protocol previously described (*5*). Thyroidectomized male Sprague-Dawley rats were allowed several months to become hypothyroid. Either T_3 (70 ng per 100 g) or T_4 (800 ng per 100 g) or both were injected intravenously with [^{125}I]T_3 (specific activity, ~ 500 μc/μg) or [^{125}I]T_4 (specific activity, ~ 6000 μc/μg)—15 and 150 μc per 100 g, respectively. [^{131}I]Albumin was injected simultaneously with [^{125}I]T_4 to allow corrections for the plasma contribution to tissue radioactivity [(*6*); see also (*12*) below]. All animals received 2 mg of NaI intraperitoneally to dilute the $^{125}I^-$ pool. Animals were killed at indicated times by aortic exsanguination. Rats injected with T_4 were then perfused with 20 to 25 ml of cold 0.15*M* NaCl to minimize trapped plasma in the tissues. Anterior pituitaries were removed and weighed, and nuclei were prepared essentially as described elsewhere (*9*). Recovery of pituitary DNA was 6.5 to 7.5 mg/g. The nuclei were intact and uncon-

taminated by other subcellular organelles, as seen under phase-contrast microscopy. Plasma radioactivity was determined after precipitation with trichloroacetic acid (TCA). Portions of the extranuclear fraction and whole nuclear pellet were also counted. The amount of $[^{125}I]T_3$ contaminating $[^{125}I]T_4$ and plasma $[^{125}I]T_3$ in animals injected with $[^{125}I]T_4$ was measured by affinity chromatography, using specific T_3 antibody conjugated to Sepharose, as described elsewhere (*10*). Nuclear radioactivity was analyzed by paper chromatography in *tert*-amyl alcohol (TAA), hexane, and ammonia (*11*) and *n*-butanol saturated with 2*N* NH_4OH after extraction with butanol and ammonia (99 : 1) (recovery was greater than 90 percent). The $[^{125}I]T_3$ was identified by its precise cochromatography with $[^{131}I]T_3$ (Amersham/Searle) in both chromatographic systems, and its cochromatography with nonradioactive T_3 stained by diazotized sulfanilic acid in both systems. The separation of T_4 and T_3 peaks in the TAA-hexane-NH_4OH chromatographic system was between 12 and 15 cm, and this system can resolve T_3, T_4, reverse T_3 (3,3′5′-triiodothyronine), and 3,3′-diiodothyronine (*11*). The nuclear and plasma contents were calculated from the specific activity of the dose with appropriate corrections for the known percentage contamination of T_4 with T_3 when T_4 was injected (*12*). Nuclear radioactivities were reduced to less than 2.3 and 1.5 percent of those found with tracer T_3 and T_4 injections, respectively, when nonradioactive T_3 (20 μg per 100 g) was given. Thus, nonspecific binding of $[^{125}I]T_3$ was considered to be negligible. Serum TSH was measured in samples collected before injection of iodothyronines and at the indicated times, using materials and methods provided by the Rat Pituitary Hormone Distribution Program of the National Institute of Arthritis, Metabolism, and Digestive Diseases.

The temporal relationship of plasma T_3 and TSH and the pituitary nuclear T_3 content after T_3 injection are depicted in Fig. 1. Virtually all radioactivity bound to the pituitary nuclear receptor cochromatographed with authentic T_3. There is a remarkable inverse correlation between nuclear occupancy and serum TSH concentrations. The interval between the maximum nuclear T_3 concentration and the minimum plasma TSH concentration is about 1 hour. Considering that the half-time for TSH disappearance in thyroidectomized rats is 30 minutes (*13*), the decrease in TSH to 59 percent of the baseline value ($P < .001$) at 2 hours indicates a very short delay between nuclear occupancy and inhibition of TSH release. From the half-time of disappearance and the nadir of serum TSH it is possible to estimate that the TSH secretion has been suppressed to approximately 50 percent, which is in good agreement with the maximum occupancy of 45 percent based on our estimates of the maximum binding capacity for T_3 in pituitary nuclei from these animals (0.96 ng of T_3 per milligram of DNA). The direct relationship between nuclear T_3 receptor saturation and inhibition of TSH release is further supported by the fact that 630 ng of T_3 per 100 g resulted in 96 percent saturation of receptors and maximum suppression of plasma TSH (not different from that produced by 20 μg of T_3 per 100 g). The relationship is not completely linear, since at 7 hours TSH has returned to basal levels and there is still significantly more nuclear T_3 than there was before T_3 injection ($P < .025$). It is of interest that the nucleus/plasma T_3 ratio from 2 hours on is approximately 0.65, which is quite similar to that obtained by Oppenheimer *et al.* (*9*) in euthyroid animals.

When 800 ng of $[^{125}I]T_4$ per 100 g is injected, the percentage of the dose present in the pituitary and the pituitary/plasma ratio are significantly lower than they are after T_3 injection, which confirms previous observations (*6*). Surprisingly, 30 to 35 percent of the total tissue radioactivity was found in the nucleus. Chromatographic analysis showed that more than 90 percent of this radioactivity was due to $[^{125}I]T_3$. No significant quantities of other labeled compounds were identified. Based on the specific activity of the injected T_4, the amount of T_3 present in the nucleus at 1 and 2 hours was not different from that in animals injected with T_3 at 70 ng per 100 g (Table 1). As can be seen in the last column of Table 1, this quantity of T_4 produced the same effect on plasma TSH as did the injected T_3, which confirms our previous results (*5*). The similar responses in TSH were thus correlated with similar quantities of T_3 present on the nuclear receptors. The amount of $[^{125}I]T_3$ derived from $[^{125}I]T_4$ in the plasma of T_4-injected animals was virtually undetectable (corresponding to T_3 concentrations < 0.06 ng/ml); this resulted in a pituitary nucleus/

Table 1. Pituitary nuclear and plasma T_3 and plasma TSH in hypothyroid rats after a single intravenous injection of T_3 or T_4 or both. Nuclear and plasma T_3 are based on the specific activities of the injected iodothyronines. The values represent increments over basal levels. Basal nuclear T_3 was < 0.12 ng/mg DNA and basal plasma T_3 < 0.18 ng/ml. Each value is the mean ± S.D. The number of animals is given in parentheses. Plasma TSH values are percentages of basal levels. All 1-hour TSH values were not significantly different from basal TSH by a paired *t*-test. All 2-hour TSH values were significantly different from basal TSH by a paired *t*-test ($.05 > P > .005$).

Injection	Experiment	Nuclear T_3 (ng/mg DNA)		Plasma T_3 (ng/ml)		Plasma TSH (%)	
		1 hour	2 hours	1 hour	2 hours	1 hour	2 hours
			Group I				
T_3, 70 ng per 100 g	A	0.45 ± 0.02 (3)	0.43 ± 0.02 (4)	1.3 ± 0.1	0.82 ± 0.10	84 ± 11	59 ± 4
	B		0.43 ± 0.04 (4)		0.63 ± 0.02		50 ± 18
Mean ± 1 S.D.*			0.43 ± 0.04 (8)				55 ± 13
			Group II				
T_4, 800 ng per 100 g	C	0.36 ± 0.05 (4)	0.38 ± 0.02 (4)	< 0.01	< 0.06	90 ± 3	67 ± 16
	D		0.42 ± 0.04 (5)		< 0.01		68 ± 8
Mean ± 1 S.D.*			0.40 ± 0.04 (9)				67 ± 12
			Group III				
$[^{125}I]T_3$, 26 ng per 100 g	E	0.15 ± 0.02 (4)†	0.13 ± 0.01 (4)	0.24 ± 0.03	0.17 ± 0.01		
$[^{125}I]T_3$, 26 ng per 100 g, + T_4, 800 ng per 100 g	F	0.13 ± 0.01 (4)†	0.11 ± 0.01 (4)	0.24 ± 0.01	0.17 ± 0.03		
P values		I versus II, NS‡ E versus F, NS	I versus II, NS E versus F, NS			I versus II, NS	I versus II, NS

*Mean ± S.D. of the whole group. There was no difference between the results of the experiments within these groups. †The T_3 results refer only to the $[^{125}I]T_3$ and are based on the specific activity of the injected $[^{125}I]T_3$. ‡Not significant, $P > .05$.

plasma T_3 ratio many times that of the T_3-injected rats.

Since previous attempts to demonstrate in vivo deiodination of T_4 to T_3 in pituitary homogenates have been unsuccessful (*14*), we explored the possibility that this quantity of T_4 could facilitate the entrance of the T_3 present in the plasma into the nucleus, thus explaining the high nucleus/plasma ratio for T_3 in T_4-injected animals. Group III in Table 1 shows that this is not the case. When [^{125}I]T_3 (26 ng per 100 g) was given with nonradioactive T_4 (800 ng per 100 g), the amount of [^{125}I]T_3 present in the nucleus at 2 hours was not different from that found in rats given only T_3 (experiment E compared to experiment F). One can determine from these data that the nucleus/plasma [^{125}I]T_3 ratio at 2 hours in hypothyroid animals given tracer T_3 was 0.76 ± 0.08 (mean ± standard deviation), not different from the value of 0.65 ± 0.12 in the animals receiving tracer T_3 plus nonradioactive T_4.

The data shown in Fig. 1 support the concept that the rapid inhibitory effect of T_3 on pituitary TSH release occurs through T_3 binding to nuclear receptors. This is consistent with the results of previous studies, which showed that inhibition of protein synthesis by actinomycin D blocks the TSH-suppressive effect of thyroid hormone (*15*) and with studies of the mechanism of thyroid hormone action in other systems (*1-3*).

The results presented in Table 1 provide an explanation for the apparent direct rapid effect of T_4 on TSH suppression. When T_4 is given to hypothyroid animals, there is rapid monodeiodination within the pituitary cells (presumably including the thyrotrophs), with subsequent binding of T_3 to the nuclear T_3 receptors. This is not accompanied by the appearance of significant quantities of T_3 in the plasma. The effect of T_4 is well correlated with the nuclear content of T_3, since equal quantities of nuclear T_3 from T_3 given directly produce the same effect on plasma TSH. Since pituitary nuclear T_3 in the T_4-injected animals does not come from plasma, and Galton (*14*) has shown no conversion of T_4 to T_3 in pituitary tissue in vitro, the findings reported here seem contradictory. However, as mentioned previously, several investigators have found significant quantities of labeled T_3 in pituitary tissue or in thyrotropic tumors after injection of labeled T_4 in vivo (*8*), and Reichlin *et al.* (*16*) have suggested that an intact hypothalamic-pituitary connection might facilitate conversion of T_4 to T_3. This could be an explanation for the apparent

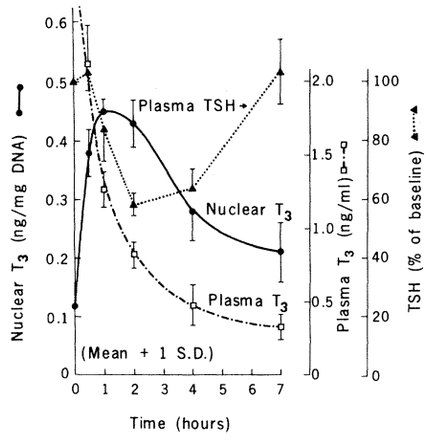

Fig. 1. Time course of pituitary nuclear T_3 and plasma T_3 and TSH after administration of a single intravenous dose of 70 ng of T_3 per 100 g to thyroidectomized rats. Each point is the mean ± standard deviation (S.D.) for four rats. The amount of T_3 was calculated from the [^{125}I]T_3 found in the nucleus or TCA-precipitable plasma and the specific activity of the dose. Nuclei were prepared as described elsewhere (*9*) and nuclear radioactivity was > 90 percent T_3 at all times. The amount of tracer not displaced by an excess of nonradioactive T_3, the nonspecific binding, was < 2.3 percent at 2 hours. Plasma TSH was measured by radioimmunoassay. Only 2- and 4-hour points are significantly different ($P < .001$) from the basal TSH concentrations by paired *t*-test. The mean ± S.D. of plasma TSH concentration at time 0 was 1236 ± 558 μU/ml (normal range, 50 to 150 μU/ml).

difference between the in vitro and in vivo results.

It must be added that, although DeFesi and Surks (*17*) recently reported that about 36 percent of the cells in the chronically hypothyroid rat are thyrotrophs, our studies do not distinguish intracellular events in thyrotrophs from conversion of T_4 to T_3 in other pituitary cells. In preliminary studies of euthyroid rats we have observed pituitary conversion of T_4 to T_3 quantitatively similar to that reported here in hypothyroid rats, which suggests that this process is not unique to the hypothyroid rat and, presumably, not to the thyrotroph.

If inhibition of TSH release is mediated by nuclear T_3 binding, then our results suggest that T_4 produces its effect only after its conversion to T_3. While our data were obtained from hypothyroid rats, it is tempting to speculate that in the euthyroid state, both plasma T_3 (largely produced from conversion of T_4 to T_3 in the liver and kidney) and plasma T_4 (through its conversion to T_3 in the thyrotroph) contribute to feedback inhibition of TSH release. The data in Table 1 show that the pituitary nucleus/plasma [^{125}I]T_3 ratio in hypothyroid rats given tracer T_3 (26 ng per 100 g) is not significantly higher than that in animals with concentrations of plasma T_3 in the physiological range. As a result of this, the contribution of plasma T_3 to pituitary nuclear T_3 does not increase significantly, except as a consequence of an increase in free T_3 in plasma. Therefore, unless there is a compensatory increase in plasma T_3, a decrease in free T_4 in plasma will result in desaturation of pituitary nuclear T_3 receptors because of a decrease in intracellular T_3 production. This decrease in the occupancy of the nuclear receptors would eventually lead to an increase in TSH release.

Such a mechanism would explain the elevated TSH found in iodine deficiency and the early phases of thyroid dysfunction in humans, in which T_4 is reduced and T_3 is normal or even slightly elevated (*7, 18, 19*). Such a dual regulatory system would also explain why an acute reduction in plasma T_3 through inhibition of conversion of T_4 to T_3 in the liver and kidney, such as is produced by PTU, could result in an acute elevation in TSH even though T_4 remains constant (*5*). That the latter effect is due to PTU-induced inhibition of peripheral, rather than intrapituitary, conversion of T_4 to T_3 is supported by our preliminary results showing no effect of PTU pretreatment on either the nuclear T_3 or the degree of acute TSH suppression in hypothyroid rats given T_4.

J. E. SILVA
P. R. LARSEN

Thyroid Unit,
Peter Bent Brigham Hospital,
Boston, Massachusetts 02115

References and Notes

1. J. H. Oppenheimer, H. L. Schwartz, M. I. Surks, D. Koerner, W. H. Dillman, in *Recent Progress in Hormone Research*, R. O. Greep, Ed. (Academic Press, New York, 1976), p. 259.
2. J. H. Oppenheimer, E. Silva, H. L. Schwartz, M. I. Surks, *J. Clin. Invest.* **59**, 517 (1977).
3. H. H. Samuels and L. E. Shapiro, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3369 (1976).
4. W. R. Ruegamer, J. S. Warren, M. Barstow, W. Beck, *Endocrinology* **81**, 277 (1964); J. H. Oppenheimer, H. L. Schwartz, M. I. Surks, *J. Clin. Invest.* **51**, 2493 (1972); R. D. Frumess and P. R. Larsen, *Metabolism* **24**, 547 (1975).
5. P. R. Larsen and R. D. Frumess, *Endocrinology* **100**, 980 (1977).
6. A. R. Schadlow, M. I. Surks, H. L. Schwartz, J. H. Oppenheimer, *Science* **176**, 1252 (1972); H. H. Samuels and J. S. Tsai, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3488 (1973).
7. H. Fukuda, N. Yasuda, M. A. Greer, *Endocrinology* **97**, 924 (1975); I. J. Chopra, D. H. Solomon, G. H. Chua Teco, *J. Clin. Endocrinol. Metab.* **36**, 1050 (1973).
8. D. H. Ford and J. Gross, *Endocrinology* **62**, 416 (1958); S. C. Werner, E. M. Volpert, R. Ginsberg, *Nature (London)* **192**, 1193 (1961); E. M. Volpert, R. Ginsberg, S. C. Werner, *Endocrinology* **71**, 361 (1962).
9. J. H. Oppenheimer, H. L. Schwartz, M. I. Surks, *Endocrinology* **95**, 897 (1974).
10. C. J. Zimmerman, M. Izumi, P. R. Larsen, *Metabolism*, in press.
11. D. Bellabarba, R. E. Peterson, K. Sterling, *J. Clin. Endocrinol. Metab.* **28**, 305 (1968); A. Taurog, G. Riesco, P. R. Larsen, *Endocrinology* **99**, 281 (1976).

12. In animals injected with [^{125}I]T_3, radioactivity from trapped plasma did not account for more than 3 percent of ^{125}I found in the pituitary. In T_4-injected animals, after washing with cold saline as described, plasma accounted for 20 to 30 percent of the radioactivity found in the extranuclear fraction and 0 to 18 percent of the nuclear radioactivity. The plasma contribution, P, was calculated as (^{131}I in pituitary fraction/TCA-precipitated ^{131}I per microliter of plasma) × (TCA-precipitated ^{125}I per microliter of serum). The amount of T_3 generated from T_4 was determined as follows. First, a correction factor for [^{125}I]T_3 in nucleus or plasma derived from [^{125}I]T_3 contaminating the [^{125}I]T_4 was subtracted. This was computed as $P \times T$, where P is the percentage of [^{125}I]T_3 in the [^{125}I]T_4 injected, and T is the fraction of injected [^{125}I]T_3 found in the nucleus (or plasma) at a particular time after [^{125}I]T_3 injection. In experiments C and D (Table 1), the correction factor was 5 to 12 percent of the nuclear [^{125}I]T_3 and 90 to 100 percent of the plasma [^{125}I]T_3. Second, the corrected [^{125}I]T_3 (derived from [^{125}I]T_4 conversion) as a percentage of the dose of [^{125}I]T_4 was then used to calculate the mass of T_3 produced (nanograms) as follows: (corrected [^{125}I]T_3/total dose of [^{125}I]T_4) × 2 × total dose of T_4 (nanograms) × (651/777). The factor 2 derives from the fact that [^{125}I]T_4 (Cambridge Nuclear) was prepared by iodinating 3,5-diiodothyronine with carrier-free ^{125}I. All T_4 molecules are therefore labeled at the 3′ and 5′ positions. Thus, each molecule of T_3 formed from T_4 has one-half the radioactivity of the original.
13. E. Silva and P. R. Larsen, in *Program of the 59th Annual Meeting of the Endocrine Society* (Endocrine Society, Bethesda, Md., 1977), abstr. 462. The half-time of disappearance of TSH is 30 minutes in thyroidectomized animals, whether measured by immunoprecipitation of injected [^{125}I]TSH or by the immunoassay of TSH after thyrotropin-releasing hormone stimulation or T_3 administration [with appropriate corrections for nonsuppressible TSH; see M. I. Surks and J. H. Oppenheimer, *Endocrinology* **99**, 1432 (1976)].
14. V. A. Galton, in *Thyroid Research*, J. Robbins and L. E. Braverman, Eds. (Excerpta Medica, Amsterdam, 1976), p. 251.
15. J. L. Bakke and N. Lawrence, *Eur. J. Pharmacol.* **2**, 308 (1968).
16. S. Reichlin, E. M. Volpert, S. C. Werner, *Endocrinology* **78**, 302 (1966).
17. C. R. DeFesi and M. I. Surks, *Clin. Res.* **25**, 462 (1977).
18. G. M. Abrams and P. R. Larsen, *J. Clin. Invest.* **52**, 2522 (1973).
19. P. R. Larsen, *Metabolism* **21**, 1073 (1972).
20. We thank T. E. Dick for his valuable technical assistance and A. Duli for preparation of the manuscript. Supported by NIAMDD grant AM 18616. P.R.L. is an investigator of the Howard Hughes Medical Institute.

2 May 1977; revised 1 July 1977

Cell-Free Modulation of Proinsulin Synthesis

Abstract. *In vivo, glucose preferentially stimulates proinsulin biosynthesis; at least part of this process is independent of new RNA synthesis and is accompanied by increases in the overall rate of polypeptide chain initiation. The cell-free translation of proinsulin messenger RNA is very sensitive to changes in the protein-synthesizing system. Proinsulin synthesis is preferentially inhibited by the addition of increasing quantities of polyadenylate-containing RNA from the fetal bovine pancreas or by the addition of the drug, aurintricarboxylic acid, which blocks polypeptide chain initiation. These results suggest that proinsulin messenger RNA competes less efficiently for rate controlling initiation factors. We propose that glucose stimulates proinsulin biosynthesis by allowing the less competitive proinsulin messenger RNA to be translated more efficiently.*

In slices of pancreas, insulinomas, or isolated pancreatic islets, it appears that glucose (and certain other metabolites) will stimulate the synthesis of proinsulin, relative to other proteins synthesized within the beta cell (*1*-*5*). For example, in studying [^{3}H]leucine incorporation into rat islet proteins, Permutt and Kipnis (*2*) demonstrate that proinsulin comprises 6.1 and 21.8 percent of the radioactive protein synthesized during incubation with low (2.8 m*M*) and high (15.3 m*M*) concentrations of glucose, respectively. This stimulation is accompanied by a twofold increase in total protein synthesis and is independent of new RNA synthesis (*2*, *3*). Furthermore, Permutt (*4*) has determined that the glucose stimulation brings about a twofold increase in the overall rate of polypeptide chain initiation, but does not alter the overall rate of chain elongation or the total amount of messenger RNA (mRNA) available for translation. There are some data compatible with glucose regulation at the transcriptional level, but clearly translational modulation is present. The mechanisms responsible for the translational control of proinsulin synthesis remain undefined.

We have studied the translation of the mRNA for bovine proinsulin in a cell-free protein-synthesizing system derived from wheat germ (*6*) and have found that it is possible to modulate proinsulin synthesis by changing the conditions of translation. These results suggest that proinsulin mRNA competes less efficiently for rate controlling initiation factors. This finding suggests that, in vivo, increases in the overall rate of polypeptide chain initiation modulate proinsulin biosynthesis in accordance with Lodish's kinetic model for translational regulation (*7*).

For the experiments described here, RNA containing a polyadenylate [poly(A)] sequence at its 3′ end was isolated from the fetal bovine pancreas (*6*) and translated in the wheat germ system as described by Roberts and Paterson (*8*). A sensitive double antibody immunoprecipitation technique (*6*) was used to quantitate the radioactive amino acids incorporated into preproinsulin (*6*, *9*). Figure 1A shows that the optimum Mg^{2+} concentration for proinsulin synthesis in vitro was 1 m*M* lower than the optimum for total protein synthesis. Under conditions in which small variations in the Mg^{2+} concentration had no effect on total protein synthesis, proinsulin synthesis was severely depressed. This phenomenon was observed consistently at all mRNA concentrations and with different combinations of wheat germ lysates and mRNA samples. Increases in the K^+ concentration also appeared to inhibit selectively the synthesis of proinsulin (Fig. 1B). Figure 1C shows that at concentrations of mRNA below 1 μg per 50-μl assay, total protein and proinsulin were synthesized proportionally. However, above 1 μg/50 μl (Fig. 1, C and D) the synthesis of proinsulin was depressed.

Other investigators have been able to modulate different proteins synthesized in cell-free systems by varying mRNA competition (*10*–*12*), polyamines (*13*), and ionic conditions (*14*). Many other investigators use low background cell-free systems with the implicit assumption that the resultant translation products reflect the true complexity and abundancy of the input mRNA population. This concept has not been directly proved. Since it is so easy to change the percentage of the translation products that react with proinsulin antiserums, it is difficult to determine the proportion of the mRNA population coding for proinsulin. These results argue against the indiscriminate extrapolation of cell-free translation data to estimate mRNA concentration.

Our results are similar to those obtained by McKeehan (*11*) in a study of globin chain synthesis in a reconstituted translation system. He found that the concentrations of mRNA, ribosomal subunits, different initiation factors, Mg^{2+}, and K^+ all affect the ratio of α- to β-globin synthesized. Lodish (*15*) and Lodish and Jacobson (*16*) had previously shown that β-globin mRNA initiates polypeptide chain synthesis more efficiently than α-globin mRNA. McKeehan (*11*) and Lodish (*7*, *15*) conclude that α-globin mRNA has a lower affinity for active 40*S* ribosomal subunits.

Our results can be interpreted in a similar fashion. At low concentrations of mRNA, the competition for rate controlling elements is less severe and the proinsulin mRNA is efficiently initiated. With increasing quantities of mRNA the translational apparatus becomes saturated and less proinsulin is synthesized. Apparently, those mRNA's that are preferentially translated possess higher affinities for the relevant rate controlling ele-