Time Course of Thyroid Response to Rising and to Falling Blood Levels of Thyroxine.*† (27068)

STANLEY LANG AND SEYMOUR REICHLIN‡

Surgical Laboratories, The Jewish Hospital of Saint Louis, and Depts. of Medicine, Preventive Medicine and Psychiatry, Washington University School of Medicine, St. Louis

Feedback control of pituitary thyrotrophin release by thyroxine has been amply demonstrated (cf.1). Although the inhibitory effect of thyroxine may be exerted in part through the hypothalamus(2), there is good evidence to show that this hormone regulates TSH output primarily by a direct action on pituitary tissue (cf.3). Little is known of the relationship between blood thyroxine concentration and rate of TSH secretion; still more obscure is the cellular mechanism by which thyroxine exerts its regulatory effects on the pituitary. In an attempt to define this problem more precisely, experiments to determine the time course of pituitary-thyroid response to increase in thyroxine concentration and to falling blood thyroxine levels were carried out.

Methods. This study was done in two parts. The first, utilizing rabbits, was carried out several years ago and the second experiment, utilizing rats, was more recently completed.

A. *Inhibitory response to thyroxine injection.* The technique for measuring thyroid activity in the rabbit and conditions of housing and diet have been recorded(4). In the present experiment 3 rabbits were used to measure accurately the latent period of thyroid inhibition following intravenous thyroxine injection. Twice daily neck counts of thyroid radioactivity were made beginning 48 hours after intraperitoneal injection of 40 μc of NaI^{131}. From the time of the first count 20 mg of methyl thiouracil were administered subcutaneously twice daily to block the uptake and reutilization of radioiodine from degraded endogenous I^{131}-labeled thyroxine. The use of the goitrogen also caused a marked acceleration of thyroid I^{131} release rate which permitted greater precision in measurement of the time at which thyroid inhibition occurred. Following an initial observation period of 3 days, 250 μg of l-thyroxine dissolved in 2 ml of slightly alkaline physiological saline were injected intravenously at an accurately noted time. Five additional neck counts were obtained over the next 3 days. The time of onset of inhibition was estimated from the intersection of the control release curve with the backward extrapolated curve of the thyroxine period (Fig. 1). Continued thyroidal inhibition was ensured by giving an additional dose of 100 μg of thyroxine one day later.

B. *Release of thyroid inhibition as blood thyroxine levels fall.* The principle of this experiment was to follow serially plasma PBI levels as they fell after thyroxine injection, and to correlate these values with measurements of thyroid gland activation as determined by serial changes in plasma PBI^{131}.

Male Holtzman rats of the Sprague-Dawley strain, 250 to 400 g in weight were used for this experiment. They were housed individually in a controlled temperature room and were fed Purina Laboratory Chow *ad libitum.* On the first day of experiment the rats were injected with 20 μc of NaI^{131} i.p. Exactly one day later, 20 μg of l-thyroxine were similarly injected. At intervals thereafter groups of 8 rats each were sacrificed under ether anesthesia by bleeding from the carotid artery through a polyethylene cannula into heparinized tubes. Chemical PBI was determined by the method of Chaney, *et al.*(5) on plasma from individual rats. Total and 10% trichloracetic acid precipitable radioactivity were determined in a scintillation well counter. Appropriate corrections for radioactivity decay were made.

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‡ Present address: Endocrinology Unit, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

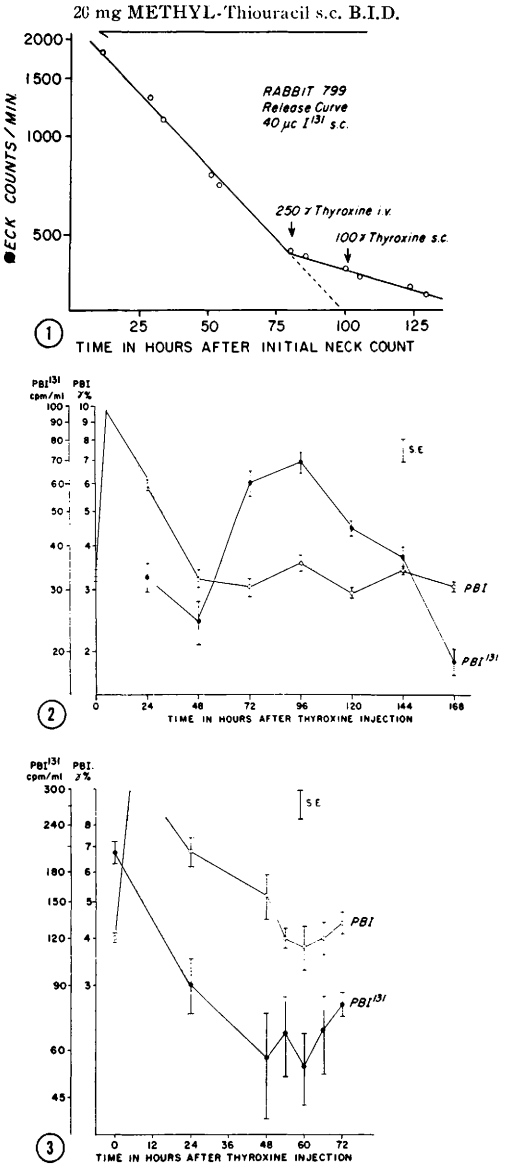

FIG. 1. Effect of intravenous injection of thyroxine on I^{131} release from the thyroid gland of a rabbit measured by external counting. Methyl-thiouracil was injected to prevent I^{131} recirculation and to increase rate of activity of pituitary-thyroid axis. Time of inhibition is coincident with time of injection.

FIG. 2. Change in blood protein bound iodine (PBI) and trichloracetic acid insoluble plasma radioactivity (PBI^{131}) which follows injection of 20 μg l-thyroxine i.p. in groups of rats (8 rats per time interval) pre-treated with NaI^{131}. Radioiodine was injected 24 hr before start of experiment. At 0 time thyroxine was injected and between 24 and 48 hours PBI continued to fall, leveling off between 48 and 72 hours. Marked activation of pituitary-thyroid axis occurred betwen 48 and 72 hours as measured by concentration of PBI^{131}.

Results. A. *Latent period of thyroid inhibition following thyroxine injection.* Following intravenous injection of l-thyroxine, a prompt slowing of release of I^{131} from the thyroid gland occurred in each of the 3 rabbits (Fig. 1). Release rates fell in the 3 rabbits respectively from 41 to 8% per day, from 64 to 15% per day and from 39 to 13% per day. The time of onset of the inhibitory effect was estimated from the interception of the pre- and post-injection slopes. From these intercepts the onset of inhibition of release was observed to be coincident with time of thyroxine injection in 2 rabbits and 3 hours after injection in the third rabbit.

B. *Release of thyroid inhibition as thyroxine levels in the blood fall.* In the first group of rats studied observations were made at 24 hour intervals for 7 days after injection of thyroxine (Fig. 2). Mean plasma PBI had fallen to control levels by the 48 hour specimen and remained at control levels over the next 5 days. Plasma precipitable radioactivity which reflects the release of preformed I^{131}-labeled l-thyroxine was still falling between 24 and 48 hours. Between 48 and 72 hours (at a time when falling PBI concentration had leveled out), there was a sharp increase in plasma protein bound radioactivity, indicating thyroid activation. The interval 48 to 72 hours was explored in more detail in a second experiment by making measurements every 6 hours (Fig. 3). PBI concentration fell up to 54 hours after thyroxine injection, then leveled out sharply at values not significantly different than the normal. Thyroid activation as measured by PBI^{131} increase occurred prior to time of inflection of the curve of PBI concentration, i.e., between 48 and 54 hours which corresponds to plasma PBI levels falling from 5.2 μg% to 4.0 μg%.

Discussion. The brief duration of the latent period of pituitary-thyroid responses to al-

FIG. 3. Change in blood protein bound iodine (PBI) and trichloracetic acid insoluble plasma radioactivity (PBI^{131}) which follows injection of 20 μg of l-thyroxine i.p. in groups of rats (8 rats per time interval) pre-treated with NaI^{131}. Radioiodine was injected 24 hr before start of experiment. Falling PBI level stabilizes between 54 and 60 hours and activation of pituitary-thyroid system occurs between 48 and 54 hours when PBI values are falling from 5.2 to 4.0 μg%.

tered blood thyroid hormone levels has been brought out in these experiments and emphasizes the point made previously that this system is not as sluggish as traditionally considered(4). The latent period of thyroid inhibition following thyroxine injection involves 2 components: inhibition of TSH release and the dying away of thyroid stimulation from TSH already present in the blood. The latter time has been measured in the rabbit(6), and a "half-time" of TSH effect on I^{131} release of 70-100 minutes determined. In this experiment times of onset of thyroid inhibition no longer than 3 hours were observed. Considering the lag before TSH effects on I^{131} release wear off, it must be concluded that thyroxine acts to stop TSH secretion within one hour, and probably within a few minutes after injection. A direct effect of thyroxine on the thyroid gland can be excluded by previous studies in the rabbit(7).

In the second group of experiments plasma PBI has been taken as a measure of plasma thyroxine concentration even though it is highly likely that it is the free thyroxine level (8), and not the bound which is responsible for the physiological effects on the pituitary. Nonetheless, the bound form is in equilibrium with the free; changes in PBI will reflect changes in the physiologically active hormone. Response to falling thyroxine concentration is governed by the critical level of active hormone which triggers TSH secretion. Components in the measured change in thyroid activation are the latent period of the pituitary itself and the time it takes for TSH to elicit a measurable change in plasma radioactivity. Recent studies of Söderberg(9) indicate that bound I^{131} discharge after TSH can be detected in thyroid venous blood within 15 minutes(1). The thyroid lag therefore is quite short. Neither the critical thyroxine concentration nor the pituitary activation lag are known however, and their calculation in the present experiment is not possible because one or the other datum is essential for the precise interpretation of the curves. It is reasonable to conclude, however, that TSH release is activated as the PBI is falling from 5.2 to the normal level of 4.0 μg%, and that the critical level for activation probably lies between these values. Reinstitution of TSH secretion is probably accomplished within 6 hours after the critical thyroxine concentration is achieved.

Summary. In a study of the relationship between blood thyroxine concentration and thyrotrophin (TSH) secretion, the time course of thyroid response to thyroxine injection has been examined. As measured by thyroid I^{131} release curves pituitary-thyroid inhibition occurred immediately following sudden increase in blood thyroxine concentration in 2 rabbits and within 3 hours in a third. Groups of rats were pre-treated with radioiodine and thyroid inhibition produced by injection of thyroxine. Protein bound iodine (PBI) levels in the blood were followed serially as the inhibitory effects of the injected material gradually decreased. Pituitary-thyroid activation (as measured by sharp increase in PBI^{131}) was observed as PBI levels fell from 5.2 to 4.0 μg% (control PBI 4.0 μg%) over a 6-hour period.

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