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Constant Relation Between Blood Level of Thyrotropin and Total Thyrotropin in Thyroidectomized Mice. (26361)

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Several reviews concerning regulation of thyrotropin (TSH) release have recently appeared(1-4). A dual control seems to exist. One is a fundamental, non-neural regulatory mechanism, while the other is a neural mechanism for emergency release of TSH.

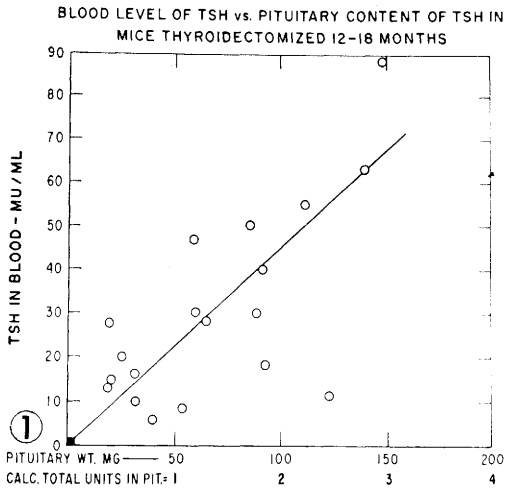
This paper presents quantitative data concerning the non-neural release of TSH. Levels of TSH in blood and pituitary or tumor were determined in two kinds of radiothyroidectomized mice, (1) those with enlarged pituitaries only, and (2) those with transplantable, TSH-producing tumors of pituitary origin. In both cases the TSH level in the blood was proportional to total amount of TSH in the pituitary or the tumor.

Methods. All mice were of the C57Bl strain and were radiothyroidectomized at about 3 months of age using approximately 200 μc of I^{131}. The 2 transplantable pituitary tumor lines 77D and 101D were obtained originally from Dr. Jacob Furth(5) and were carried for 11 and 7 transfer generations, respectively. For each transfer, suspensions of tumor tissue were injected subcutaneously into the hind leg of mice, radiothyroidectomized about one month previously. Tumors weighing 2 to 8 g developed after 6 or more months.

TSH was measured in baby chicks by thyroidal I^{131} depletion(6). Potencies are in terms of USP units. All pituitary and tumor tissues were frozen as soon as possible after removal and then lyophilized and milled to a 60 mesh powder in a Wiley mill. A suspension of this powder in 0.1 M $NaHCO_3$ was injected into chicks for estimation of potency. Heparin was added to all blood samples. Samples of blood ($>$ 1 ml) obtained from the vena cava of mice with tumors were pooled, centrifuged and the plasma used for bioassay. Blood samples, ($<$ 1 ml) obtained from individual mice with enlarged pituitaries, were assayed directly.

Results. Blood level of TSH in thyroidectomized mice with large pituitaries. The pituitaries of mice become greatly enlarged 12-18 months after radiothyroidectomy(7). Nineteen such mice with large pituitaries were killed. The blood was collected and assayed. The pituitaries were weighed and either assayed or transplanted to start new tumor lines. The positive relationship between pituitary weight and concentration of TSH in the blood is shown in Fig. 1. Most of the pituitaries that were assayed had a potency between 0.1 and 0.2 U of TSH per mg of dry pituitary weight. Hence, a unitage scale has been added to facilitate comparison with Fig. 2. The small square at the origin represents values estimated for TSH levels in normal mice (about 1 mU/ml of blood and 40 mU/2 mg of pituitary).

The milliunits of TSH per ml of blood



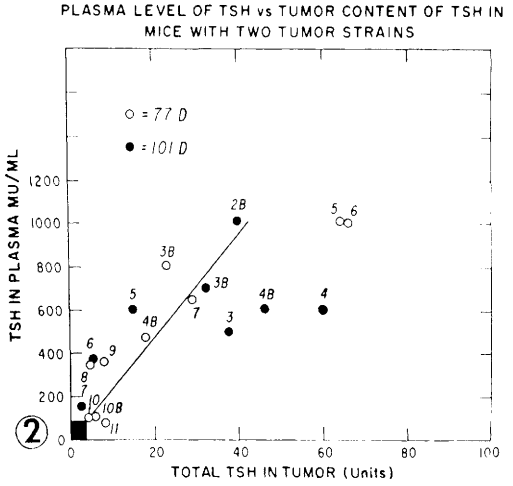


FIG. 1. Relationship of blood level of TSH to pituitary size and TSH content in mice thyroidectomized 12-18 mo. The line has a slope of 25 mU/ml/unit of TSH in the pituitary. Each point represents data from one mouse. Square at origin represents data from normal mice.

FIG. 2. Relationship of plasma level of TSH to TSH content of two strains or lines of tumors, 77D and 101D. Square at origin represents area covered by data in Fig. 1. Each point represents data obtained on pooled plasma and pooled tumor tissue from many mice. Numbers refer to transfer generation of the tumor. The line has a slope of 25 mU/ml/unit in tumor.

was divided by the calculated TSH content of the pituitary in units for each of the 19 mice. The average of these potency ratios was 25.6 ± 4 mU/ml of blood/unit of TSH in the pituitary. A line with this slope of 25 mU/ml/unit in the pituitary has been drawn in Fig. 1 to represent the average relationship found.

Plasma level of TSH in mice with TSH-producing tumors. In Fig. 2 the milliunits of TSH/ml of plasma from tumor-bearing mice are plotted against total amount of TSH in tumors of average size for each of several generations. Each point represents data from assays of a pool of plasma and a pool of tumors from many mice. The potency of the tumors decreased markedly in the later generations. As the potency of the tumors decreased, so did concentration of TSH in the blood. The small rectangle at the origin covers the area of this graph which would include the data presented in Fig. 1. A line has been drawn which has the same apparent slope as the one in Fig. 1. A curved line might fit the points better, but there is considerable scatter. The mean potency ratio for the 19 different tumor generations was 33 ± 3.5 milliunits per ml of plasma per unit of TSH in the tumor.

The above data were from old tumor lines that had already lost much potency by the time tests were begun. New tumor lines are now being established in our laboratory. These vary greatly in potency. Tumors of the first transfer of our tumor line W were especially potent. Three mice with this tumor had 5 g tumors having a potency of 0.23 U/mg dry weight or 230 U/tumor. Plasma level of TSH was 14 U/ml. The potency ratio $\left(\frac{14{,}000}{230}\right)$ obtained in these mice was 61 mU/ml of plasma/unit in the tumor, or about 30 mU/ml of blood/unit in the tumor. This is very similar to the ratio of 25 found in the mice without tumors. If plotted, this point (230 U/tumor × 14 U/ml blood) would require a graph 10 times the size of Fig. 2, or to another order of magnitude larger. The potency of lyophilized plasma from these animals was 0.14 U/mg, a most remarkable potency because it is equal to TSH concentration in the pituitary of the normal mouse and of most tumor tissues per milligram dry weight.

Inhibition of TSH production by thyroxine. Several mice with large TSH tumors were injected with 10 μg of L-thyroxine a day for 4 days. In all cases the blood level of

TSH dropped to 10-30% of the starting level. One mouse with a 1 g tumor survived daily injections of 10 μg of thyroxine for 72 days, when it was killed. During this time the tumor did not change in size. Plasma TSH level at death was still 40 mU/ml. This was 4% of the TSH level in the same mouse before thyroxine injection, but was 40 times the blood level in normal mice. Also, the concentration of TSH in the tumor was found to be 4% of that in other tumors from mice of the same generation that were not treated with thyroxine. The potency ratio after thyroxine for 72 days was 30 mU/ml of blood/unit in the tumor.

Effect of TSH level on hypertrophy of the pituitary. The high blood level of TSH in mice with tumors does not prevent the hypertrophy of the pituitary that begins about a year after thyroidectomy. Tumors of strain 101-D, generation 4, grew so slowly that the tumors of 19 mice were not harvested until 11 months after thyroidectomy, about 10 months after transfer. Average pituitary weight of these 19 mice was 9.8 mg, which is more than 5 times the normal weight of the pituitary. TSH concentration in the pituitaries was 0.1 U/mg dry weight, which is essentially normal. These data clearly show that the blood level of TSH does not inhibit the factor or factors causing pituitary hypertrophy in thyroidectomized mice. The physiological inhibiting agent is known to be thyroid hormone(8,9), produced by the thyroid and obviously absent in these thyroidectomized mice.

Discussion. It is of interest that the relation between blood level of TSH and total amount of TSH in the body of these thyroidectomized mice varied on the average by a factor of not more than 2, whether the mice were treated with thyroxine or not and whether the primary source of the TSH was the pituitary *in situ* or a tumor in the leg, even though the level of TSH in the plasma ranged from 1 to 14,000 mU/ml. Thus in thyroidectomized mice the blood level of TSH reflects almost quantitatively the total stores of TSH in the body, whether in the pituitary or in a tumor in the leg. This relation, of course, may be altered temporarily in a normal mouse by hypothalamic stimulation. But in the case of the mice with tumors in the leg the release of TSH is surely independent of hypothalamic control.

No appreciable storage of TSH in body tissues outside of the pituitary gland and pituitary tumors has been found(10). The blood level of approximately 25 mU/ml/unit of TSH in the pituitary or tumor, when viewed conversely, indicates that the blood level of TSH in U/ml multiplied by 40 gives an estimate of total units of TSH stored in the pituitary or tumor tissue of these mice. Since the blood volume of a mouse is less than 3 ml, it follows that 90-95% of total TSH in the body is estimated to be in the pituitary or tumor tissue.

The pituitary is still of normal size (<2 mg) in most mice with tumors, so that the 20 to 40 milliunits present in the pituitary contribute very little to the total amount of TSH in the body. In a mouse with a tumor the total amount of TSH in the blood (about 1 unit) is usually less than 5% of that in the tumor, but some 50 times that in the pituitary.

Kumaoka, Money, and Rawson(11) have recently studied the inhibitory effect of thyroxine and thyroxine analogues upon growth of TSH-producing tumors similar to those used here. Combining their studies with those reported here, it can be stated that in mice with TSH-producing tumors, thyroxine injections at a high dosage level(1) inhibit or stop tumor growth; (2) lower TSH levels in blood to about 5% of the initial level, but not to normal levels, and (3) lower TSH concentration in the tumor to about 5% of initial concentration, but not to zero.

Summary. In thyroidectomized mice approximately the same relationship exists between the blood level of TSH and total amount of TSH in the TSH-producing pituitary tissue in the body whether this tissue is *in situ* in the sella turcica or in a tumor in the hind leg, and while the amount of TSH in this tissue varies by 10,000 times from an amount of 20 milliunits to 200 units. These results demonstrate clearly and quantitatively the autonomous release of pituitary TSH independent of any hypothalamic in-

fluence. In these mice the units of TSH/ml of blood times 40 gives an approximate measure of total TSH in units in the body. Thyroxine treatment caused a parallel lowering of the level of TSH in blood and tumor.

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Identification of Extrahepatic Bilirubin Monoglucuronide and Its Conversion to Pigment 2 by Isolated Liver. (26362)

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Bollman and Mann(1) noted that as the amount of serum bilirubin increased after hepatectomy in dogs, the van den Bergh reaction changed from indirect to direct, and bile pigment began to appear in the urine. This indicated that the liver was not the only organ capable of forming bilirubin or converting it to direct bilirubin. Later, work by Billing and Lathe(2), Schmid(3), Talafant(4) and Schachter(5) indicated that conjugation of bilirubin prior to its excretion involves solubilization and accounts for the nature of the reaction of the pigments in the van den Bergh test. The indirect reaction occurs with the water-insoluble free bilirubin, whereas the direct reaction comprises pigment 1 and pigment 2, the water soluble conjugates of bilirubin. Pigments 1 and 2, as obtained by reversed phase partition chromatography, have been described as bilirubin monoglucuronide and diglucuronide, respectively. However, other nonglucuronide conjugates of bilirubin also may be found in pigment 1 and 2 fractions. Bollman(6) observed that the direct pigment in the plasma of the hepatectomized dog has the characteristics of pigment 1 with respect to chromatographic mobility and diazotization reaction. Whether this pigment is a glucuronide or some other conjugate of bilirubin had not been established(7). The present report determines this conjugated pigment of extrahepatic origin to be bilirubin monoglucuronide.

Methods and procedures. Total hepatectomy was performed as previously described by Grindlay and Mann(8), the dog being maintained postoperatively by a saline-glucose solution administered by a constant injection apparatus. Blood was collected for analysis of the plasma pigments at 42 hours after complete removal of the liver.

The proteins were precipitated from the plasma using ethyl alcohol for 30 minutes. After centrifugation, the supernatant was evaporated *in vacuo* and aliquots were dissolved in 0.5 ml of the mobile phase of the solvent system used in the chromatographic separation. It was noted that, as found by Hoffman and associates(9), when ammonium sulfate was used in addition to ethanol for precipitation of the proteins from the plasma of the liverless dog, the pigment appeared to remain bound to the ammonium sulfate crys-