the 5 monkeys (620± 110 ng/g wet weight, Ab-IIIb) greatly exceeded that in liver of intact animals, when the total mass of the organs involved is considered, there is more iCT in the liver than in the thyroid (figure 2). Basal serum iCT was undetectable (< 20 pg/ml) in both thx and intact monkeys. Pentagastrin-calcium infusion resulted in an increase in thx monkey serum iCT values (Ab-IIIb) which was less than for intact animals (peak of 45± 50 pg/ml vs 104± 104 pg/ml). The basal urine iCT of intact and thx monkeys was detectable after boiling 5 min, concentration (lyophilization of 300 ml), extraction with NH_4OH and acetonitrile, and chromatography[5] (intact, Ab-IIIb - 97 pg/mg creatinine, thx, Ab-IIIb - 6 pg/mg creatinine).

Discussion. We have found that iCT is not confined to the thyroid gland. Undoubtedly, some of the iCT (such as that in the liver, muscle, or kidney) is receptor-bound hormone. The liver iCT decreased strikingly post thx. However, all tissues studied continued to have significant amounts of iCT 2 months after thx. Calcitonin is synthesized by C cells, which are believed to arise from the neural crest and migrate to the last branchial pouch and, subsequently, in mammals, to reside in the thyroid gland. These cells are part of a diffuse system of so-called APUD cells (staining characteristics: amine content and/or amine precursor uptake and decarboxylation)[7]. It seems likely that extrathyroidal iCT is the result of iCT secretion by these cells. In this regard, we have recently reported finding iCT in the bronchial K cells (Kultchitzky, enterochromaffin, argyrophil or Feyrter cells) of man[8]. Thus, the iCT content in any specific tissue of the thx animals could be the result of extrathyroidal secretion within that tissue, or receptor iCT which emanated from another extrathyroidal tissue, such as lung. Experiments implying a relative unimportance of iCT in the control of calcium metabolism are based upon the assumption that the thx animal is calcitonin-free; it would appear that such assumptions require critical re-examination.

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Pituitary response to gonadotropin-releasing hormone in diabetic male rats[1]

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Summary. The rise in serum luteinizing hormone concentration after treatment with gonadotropin-releasing hormone was less in diabetic castrated male rats than control castrates. In intact male rats, gonadotropin-releasing hormone treatment resulted in higher serum luteinizing hormone concentrations in diabetic than in control rats.

Diabetes mellitus seriously impairs reproductive function in male rats. Following experimental induction of diabetes, animals characteristically display reduced sexual behaviour[2], low fertility[3], reduced accessory sex gland weight[3-6], reduced numbers of interstitial cells in the testes[3], degenerative changes, in seminiferous tubules[7-9] and low serum levels of testosterone[3,5]. At least some of these changes could be related to reduced serum levels of luteinizing hormone (LH)[3,5].

Since glucose metabolism in anterior pituitary tissue is insulin-dependent[10], it is possible that the pituitary glands of insulin deficient rats are unable to respond adequately to gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus. The following experiments were conducted to test this hypothesis.

Materials and methods. Male Sprague-Dawley rats from our own colony were made diabetic at 3 months of age by an injection of streptozotocin (7 mg/100 g b.wt) into a tail vein. The induction and persistence of diabetes was confirmed by observations of glycosuria, polyuria and polydipsia.

In the 1st experiment, castrated rats were used. The testes were removed on the day before streptozotocin was injected. After 21 days, control and diabetic animals were lightly anesthetized with ether, a blood sample was obtained by heart puncture and GnRH (40 ng/100 g b.wt, in saline) was injected into a tail vein. Rats were decapitated 10 min after the injection and trunk blood was collected.

In a 2nd experiment, intact control and diabetic male rats were used on 3 occasions beginning 2 months after induction of diabetes. On each occasion rats were anesthetized with ether and injected via a tail vein with 500 ng GnRH/100 g b.wt. Rats were again anesthetized and a blood sample was obtained from the orbital sinus (first 2 occasions) or heart 10 min after GnRH injection.

In each experiment the sera obtained from the clotted blood samples were frozen and subsequently assayed[11] for LH concentration. The data were subjected to analysis of variance.

Table 1. Effect of diabetes on body weight and response to GnRH in castrated male rats (11 rats/group)

Group	Body weight (g)**	Serum LH (ng/ml) Before GnRH*	After GnRH*	ΔLH (ng/ml)*
Control	350± 19	728± 61	2507± 415	1775± 443
Diabetic	492± 10	522± 50	1402± 73	880± 64

* $p<0.05$; ** $p<0.01$; values are mean± SEM.

Results and discussion. The 1st experiment was designed to test the responsiveness of the pituitary to GnRH in the absence of the inhibitory effect of gonadal steroids. The data (table 1) indicate that diabetic rats had lower ($p < 0.05$) serum levels of LH than control rats both before and after treatment with GnRH. The reduced level of LH

prior to GnRH suggests that in diabetic rats there is some impairment in the ability to hypersecrete LH. However, the pituitary glands of these diabetic castrates were able to maintain serum levels of LH that were 10–20-fold higher than those found in intact animals (table 2). Similar data were reported in a previous study[5]. In this earlier study[5] pituitary LH concentration was measured in castrated diabetic rats and found to be not significantly different from that in castrated controls.

When treated with GnRH, the increment in serum LH level in diabetic rats was only about half that noted in controls. Although high serum LH levels were evident in both groups, the ability to secrete LH in response to GnRH stimulation was clearly impaired in the diabetic rats.

When intact rats were treated with GnRH (table 2) the diabetic animals had higher LH levels than controls ($p < 0.01$).

This was interpreted as indicating a greater release of stored hormone in response to GnRH in diabetic as compared to control rats. Pretreatment levels of LH in neither serum nor pituitary glands were obtained in this experiment. However, data from a previous study[5] indicate that diabetic rats have reduced serum levels of LH associated with elevated levels of LH in the pituitary glands, suggesting failure of release mechanisms. The LH levels after GnRH treatment were not as high as expected. The apparent loss of potency in the GnRH used was presumably due to prolonged (over 1 year) storage as a frozen solution.

The results of these experiments suggest that in the absence of gonadal steroids, the responsiveness of the pituitary gland of the diabetic rat to GnRH is impaired. Reduced serum levels of LH in intact diabetic rats[3,5], however, cannot be explained by reduced responsiveness to GnRH.

Table 2. Effect of diabetes on body weight and serum LH concentration following GnRH in intact male rats

Date	Treatment	Number of rats	Body weight (g)*	Serum LH (ng/ml)*
17 May	Control	6	579±13	48±5
	Diabetic	6	352±15	256±34
24 May	Control	6	580±12	47±6
	Diabetic	5	340±19	247±46
6 June	Control	6	600±12	29±6
	Diabetic	4	342±24	164±23

* Diabetic vs control, $p < 0.01$ for each date; values are mean ± SEM.

On the contrary, the responsiveness of the pituitary gland of the intact diabetic rat is many times greater than that of the intact control rat. A possible explanation for these findings is that the pituitary of the diabetic rat responds better because testosterone levels in diabetic rats are low[3,5]. It is known that pituitaries of castrated rats are more responsive to GnRH than those of intact rats[12] and that testosterone reduces the amount of LH released in response to GnRH[13]. If this explanation is correct then it must be assumed that the reduced inhibition by androgen is a more important factor in determining serum LH levels than the impairment of pituitary function related to insulin deficiency.

In addition these data suggest that GnRH secretion by the hypothalamus of the diabetic rat must be deficient. This conclusion is based on the assumption that normal secretion of GnRH coupled with reduced testosterone levels would lead to hypersecretion of LH. Obviously this is not the case as diabetic rats have normal or below normal serum levels of LH[3,5] but elevated levels of pituitary LH[5].

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Effect of insulin in vivo on the synthesis of free fatty acids (FFA) in chicken heart and skeletal muscle

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Summary. The effect of insulin on the synthesis of free fatty acids from glucose in the skeletal and heart muscles of chicken is examined. 10 min after glucose-(U-^{14}C) administration, labeled free fatty acids (FFA) appeared in both skeletal and heart muscles. 0.75 IU of insulin kg^{-1} b.wt significantly increased the labeled FFA at the 30, 60 and 120 min intervals, with a maximum at 60 min.

In chickens it has been difficult to demonstrate an insulin effect either in vitro or in vivo. A concentration far higher than that found in chicken plasma is always required[1–3]. Nevertheless Gomez-Capilla and Langslow[4] reported a clear effect of insulin (at physiological concentrations) on the glucose metabolism of isolated fat cells, althouht the extent of this stimulation was less pronounced than that produced in mammals.