control of bone marrow growth[7,8]. The absence of this antitheta sensitive regulatory cell (TSRC) has been suggested as a defect in the W/W^v anemic mouse[7-9]. It is difficult to assess if a similar cell is present in species other than the mouse because of the lack of information concerning alloantigen specifically present on the surface of thymocytes, and limitations on the application of the Till and McCulloch spleen colony technique[10] in species other than the mouse. Recently evidence has been presented that a suppressor T-cell might be involved in some instances of poor growth of bone marrow in T-cell depleted mice[11]. The present report describes observations of femoral cellularity in sham-operated and thymectomized rats, in thymectomized rats treated with cells from spleen, thymus, or lymph nodes, and in sham-operated and thymectomized rats implanted with a syngeneic testis.

Fischer 344 female rats were sham-thymectomized (Sham) or thymectomized (TMX) at 5 days of age using the technique of Hard[12]. For cell transfer experiments, thymus, spleen and inguinal and mesenteric lymph nodes were aseptically removed from syngeneic adult or young donors. These tissues were minced in a small volume of RPMI 1640 tissue culture medium, and then pressed through and 80-mesh wire screen. Cell counts were performed, and groups of TMX rats were injected at 14 days of age with 10^5 or 10^7 cells. For the testicular transplant experiment, at 5 days of age, groups of Sham or TMX rats were transplanted s.c. in the neck over the area of the jugular vein with 1 testis from a syngeneic, age-matched donor. All rats were maintained under carefully controlled environmental conditions until sacrifice by decapitation at 3 months of age. Right femora were removed and dissected free of surrounding tissues. The ends of the bones were removed, and contents of the marrow cavity were flushed into a vial of isotonic diluent. Red blood cells were lysed, and the marrow nucleated cellularity determined utilizing a Model Z_{BI} Coulter Counter electronic particle counter.

Results of these determinations are presented in the table. Data are expressed as 10^6 nucleated cells per femur. Compared to Sham rats, femoral cellularity was decreased in TMX rats, and increased by the addition of lymphoid cells from a variety of sources. In addition, transplantation of a testis to a Sham rat led to increased cells per femur, and this effect was enhanced even further in TMX rats with a testis transplant.

These results indicate that, in the rat, as has been previously observed in the mouse, neonatal thymectomy results in a decrease in bone marrow cellularity. This deficit in TMX rats can be corrected by administration of lymphoid cells from a variety of sources as well as by the presence of a syngeneic testis transplant. The latter effect is in good agreement with previous reports of stimulation of hematopoiesis following administration of androgens[13], of an increase in spontaneous spleen colony formation in mice treated with testosterone before irradiation[14], and of greater impairment of CFC-GM in thymectomized female as compared to thymetomized male mice[5]. These observations provide at least indirect evidence that cells from lymphoid tissues control to some degree femoral bone marrow cellularity in the rat, and that the influence of sex steroids on this process may be altered following thymectomy.

The effect of sham-thymectomy (Sham) and thymectomy (TMX), and the injection of cells or transplantation of a syngeneic testis on the number of nucleated cells per femur in Fischer 344 female rats

Treatment	Number of rats	Nucleated cells/femur $\times 10^{-6}$ (mean $\pm$ SEM)
Sham	34	57.9 $\pm$ 1.3
TMX	36	52.7 $\pm$ 1.6
TMX + cells	85	65.7 $\pm$ 0.8
Sham + testis	10	68.2 $\pm$ 6.6
TMX + testis	8	75.6 $\pm$ 8.0

$F = 14.51$; $p < 0.01$.

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Effect of dihydroergocristine infusion on tolbutamide-induced insulin secretion in man

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Summary. The insulinemic response to 1 g of tolbutamide i.v. is greatly enhanced (+ 145%) after a 60-min infusion of the α-lytic dihydrogenated ergot alkaloid, dihydroergocristine (83.3 µg/min, corresponding to a total dose of 5 mg) in 7 healthy subjects. No differences are observed in the glycemic responses.

Several drugs able to act on adrenergic receptors, consistently interfer with insulin secretion: β-adrenergic stimulating[2,3] and α-blocking[4,5] agents increase insulin secretion, whereas β-blocking[2,6] and α-stimulating[5,7] ones seem to have an inhibitory effect. The dihydrogenated alkaloids of the ergot possessing an α-blocking activity[8] have been shown to increase tolbutamide-induced insulin secretion in dog[2,11] and glucagon-induced insulin secretion in man[12]. It has recently been found that in man that a 7 days i.m. pretreatment with 0.3 mg b.i.d. of dihydroergocristine

(DHE) is able to enhance insulin response to low doses of tolbutamide[13]. This could be due either to the α-receptor blocking activity of these drugs or to a possible change in the structure of the β-cell membrane[11]. The aim of this study was to investigate the effect of an acute i.v. administration of dihydroergocristine on tolbutamide-induced insulin secretion in normal man. Dose, time and modality of DHE administration were chosen in order to obtain a complete pharmacological effect of the drug on the α-adrenergic receptors [14].

Material and methods. 7 healthy volunteers (4 female, 3 male), of mean age of 23 years, with normal response to an oral glucose tolerance test, were tested. Each subject was submitted, on 2 different mornings, to a load of 1 g of tolbutamide i.v. Prior to the second load, an infusion of 5 mg of dihydroergocristine over a 60-min period was given (83.3 µg/min). The first load was preceded by a 60-min saline infusion as a placebo. An interval of 7 days was interposed between the 2 loads. The infusions started between 8.00 and 9.00 h after an overnight fast; all subjects were on a normocaloric diet with 250 g of carbohydrates. Samples for blood glucose and insulin were drawn from a needle inserted in the antecubital vein, kept patent with NaCl solution, at the times shown in figures 1 and 2. The opposite arm was utilized for the infusion of DHE or placebo and for tolbutamide injection. Blood glucose was determined by the glucose-oxidase method (glucose analyzer) and blood insulin by the double antibody radioimmunoassay[15]. All data were evaluated according to Wilcoxon's paired t-test.

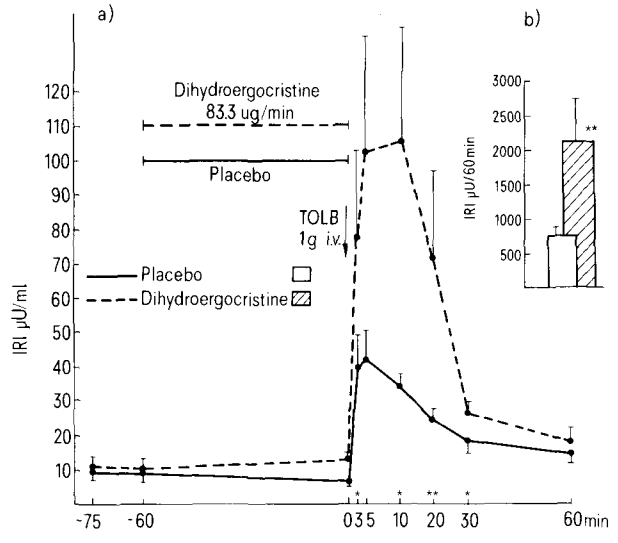

Fig. 1. Insulinemias (A) and areas of insulin secretion above the basal lines, induced by 1 g of tolbutamide i.v. in basal conditions and after a 60-min i.v. infusion of 83.3 µg/min of dihydroergocristine. Vertical bars represent ± SEM. Asterisks indicate statistical significance at $p < 0.05$ (*) and $p < 0.025$ (**) levels.

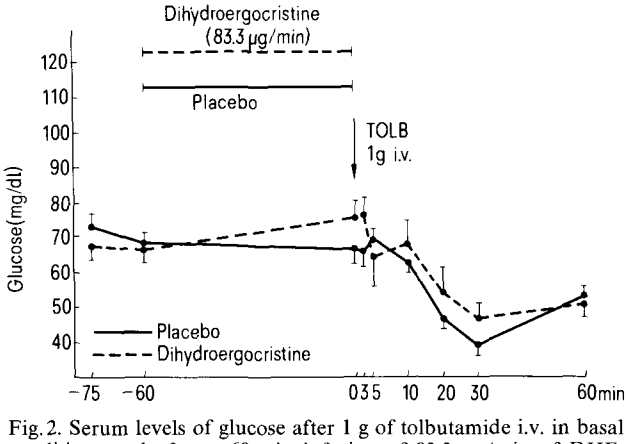

Fig. 2. Serum levels of glucose after 1 g of tolbutamide i.v. in basal conditions and after a 60-min infusion of 83.3 µg/min of DHE. Vertical bars represent ± SEM. No statistical differences were observed.

Results and discussion. DHE given by i.v. infusion over a period of 60 min greatly enhanced tolbutamide-induced insulin response. This is apparent (figure 1, A) at 3, 10, 20, 30 min ($p < 0.05$). The calculation of the areas of insulin secretion above the basal levels (figure 1, B) showed a 145% increment ($p < 0.025$) after DHE.

DHE is a dihydrogenated alkaloid of the ergot group that has a low α-mimetic intrinsic activity, in conjunction with a great affinity for the α-receptor[8]. On the contrary, nonhydrogenated ergot alkaloids that have a greater α-mimetic intrinsic activity[8] have no effect on insulin secretion[16]. Based on this data, DHE could act by inhibiting the α-receptors of the pancreatic β-cell. In fact, phentolamine, a well-known α-lytic agent, is able to increase insulin secretion either in basal conditions or after various stimuli, including sulphonylureas[7,9].

Another hypothesis on the eliciting effect of DHE on insulin secretion could be related to the ability of some ergot alkaloids to increase the cAMP intracellular content. This phenomenon has up to now been demonstrated in the brain of rat[17] and cat[18].

Sirek et al. have shown that hydrogenated ergot alkaloids influence insulin secretion induced by tolbutamide and glibenclamide, but not by arginine or glucose, in dog[10]. These authors suggested that these drugs could induce a change in the conformation of the β-cell membrane that becomes more suitable for the binding to the sulphonylureas[10]. On the basis of our experimental approach, we suggest that the ergot alkaloids, including DHE, could facilitate insulin secretion through an α-receptor blocking mechanism and by a possible increment of the β-cell content of cAMP.

Our results are in keeping with those described recently in healthy man after a prolonged i.m. administration of 300 µg b.i.d. of DHE[13]. In our approach, a greater amount of DHE (5 mg) was given by an infusion that lasted 60 min in order to obtain a true receptor-binding equilibrium[14]. In fact, it is well-documented by experiments in vitro that cellular receptors are completely saturated by some ergot alkaloids after a 30–60-min period of incubation at 37 °C[14].

The glycemic response to tolbutamide (figure 2) is not influenced by pretreatment with DHE. This fact could be due to a facilitating effect of the α-blocking drugs on glucagon secretion[4,19].

Further studies are needed to verify this hypothesis and to state the effect of DHE on glucose-induced insulin and C-peptide secretions in man.

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The adenohypophysial hormone content of the rat pars tuberalis[1]

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Summary. The pars tuberalis of the hypophysis was shown to contain LH, which increases after castration, TSH and a very low amount of PRL. FSH was found after castration.

Although the adenohypophysial rat pars tuberalis (PT) has been thoroughly studied, its function is not clear. It is mainly composed of chromophobe cells but it also contains follicular cells and a much smaller number of cells similar to the gonadotrophs of the pars distalis (PD)[2-4]. No morphological changes in the chromophobe cells were found after adrenalectomy, castration, thyroidectomy, PTU treatment, osmotic changes or modification of the calcemia[2]. Immunocytochemical studies have only shown cells positive to LH[5] and that these hypertrophy after castration[6]. Besides, hypothalamic extracts that include the PT have shown thyrotrophic activity[7].

The aim of this study was to evaluate whether the rat PT contains immunoassayable LH, FSH, PRL and TSH and compare the content of these with their level in the PD and their concentration in serum, under normal and experimental conditions that modify these hormonal levels.

Male and female adult Holtzman rats, housed under controlled conditions, and weighing 250-350 g were used. The animals were beheaded and trunk blood was collected and allowed to clot at 4 °C. Serum was then separated and frozen at −20 °C until used for hormone determination. The PT and surrounding tissue, including part of the basal hypothalamus, was dissected, taking as limits the optic chiasm, the mammillary bodies and 2 lateral cuts, each at 1 mm from the median eminence. A dorsal cut was performed as proximal to the PT as possible. The weight of the dissected fragment was about 4 mg. In this way, the PT surrounding both the median eminence and the pituitary stalk was taken. The PD was dissected using a different set of surgical instruments to avoid any contamination of the PT with adenohypophysial hormones.

The PT and PD were immediately homogenized in all-glass homogenizers containing 1% BSA in PBS pH 7.6 (50-100 µl/PT; 1000 µl/PD), centrifuged at 3000 rpm for 10 min at 4 °C. The supernatants were removed and kept at −20 °C until assayed. LH, FSH, TSH and PRL were determined by radioimmunoassay according to the NIAMDD 2nd antibody technique using as reference hormones the rat -RP_1 preparations[1].

As in some conditions we found too many values concentrated in the lower tail of the distribution curves, some of which were below the limit of sensitivity of the RIA assay, we could not assume that we had normal distributions. Therefore, we used the non-parametric Mann Whitney U test to analyse the significance of differences between groups, and thus the hormonal levels are expressed as medians.

LH was the gonadotrophin found in the highest level both in the pars tuberalis and pars distalis of female rats. After 2 weeks of castration both values increased significantly. The FSH content in the PT of the normal rat was below the limit of sensitivity for the FSH-RIA in our hands. These values were not modified after 2 weeks of castration, while the content of the PD increased significantly. The serum concentrations of both LH and FSH were significantly increased (table 1). After 3 weeks and 4 months of castration the PT content of FSH was slightly increased, becoming assayable in most cases. The median values found were 19 (n = 8) and 37 (n = 10) ng FSH/PT respectively.

The median TSH content in adult male rats (n = 30) was 237 ng/PT and 1277 µg/PD, while the serum concentration was 1700 ng/ml.

Table 2 shows that the PT of rats separated from their litters for 4 h contains PRL and that its content increases

Table 1. Median gonadotrophin values in the pars tuberalis (PT), pars distalis (PD) and serum in the female rat

	LH			FSH		
	ng/PT	µg/PD	ng/ml serum	ng/PT	µg/PD	ng/ml serum
Control	37 (30)	527 (30)	134 (30)	< 15 (19)	25 (19)	330 (19)
2 weeks castration	91* (30)	1160* (30)	407** (30)	< 15 (20)	99** (10)	1725** (20)

< 15 = lower than the sensitivity of the method. Number of animals in parentheses. * $p < 0.01$; ** $p < 0.001$.

Table 2. Median prolactin values in the pars tuberalis (PT), pars distalis (PD) and serum of the lactating rat

	Prolactin		
	ng/PT	µg/PD	ng/ml serum
4 h pup separation	0.39 (10)	99.72 (10)	32 (10)
4 h pup separation + 30 min suckling	1.14* (10)	153.16 (10)	420** (10)

For symbols see Table 1.