and the plasma assayed for corticosterone content using the microfluorometric method of GLICK et al.[4]. The results are shown in Figure 1. Up to day 10 of pregnancy, the stress response is similar to that of non-pregnant mice. After day 10, there is a progressive increase in plasma corticosterone levels during stress, reaching a 10-fold increase over non-pregnant stress levels by day 16.

In the second series of experiments mice were injected s.c. with 40, 160 or 640 mIU of ACTH (Acthar Corticotrophin, Armour Pharmaceuticals, England) on one of days 8, 11 or 16 of pregnancy after suppression of the pituitary-adrenal axis with dexamethasone (0.4 mg i.p.) 20 h before the injection of ACTH, followed by pentobarbital (80 mg/kg i.p.) plus chlorpromazine (20 mg/kg s.c.) 0.75 h before injection of ACTH[5]. Non-pregnant controls were also treated similarly. A control blood sample was taken from the retro-orbital sinus immediately before the injection of ACTH and a second blood sample taken 1 h later, this having been shown in preliminary experiments to be the time of the peak plasma corticosterone response to ACTH in the pregnant and non-pregnant mouse. The results are shown in Figure 2.

These show that on day 16 of pregnancy, when increases in plasma corticosterone levels in response to stress were much greater than in early pregnancy, there was also a much greater increase in plasma corticosterone levels in response to given doses of ACTH. Thus it is not necessary to propose that there is any increase in the secretion of ACTH in response to stress in the second half of pregnancy compared with the first half of pregnancy. The difference could be accounted for either by an increase in the sensitivity of the adrenal glands to ACTH or by a decreased rate of metabolism of corticosterone. This second possibility would seem to be the most likely mechanism in view of the known increase in protein binding of the hormone during the second half of pregnancy[1,2,6]. The lower maximum response to exogenous ACTH compared with stress observed in the present experiments may be due to a lower sensitivity of the adrenal gland to ACTH, which is known to occur by 24 h following chemical suppression or hypophysectomy[7].

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Plasma Fibrinogen Response in the Rat after Thyroid Stimulating Hormone Therapy

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Summary. Thyroid Stimulating Hormone (TSH) increased the levels of plasma fibrinogen in the presence or absence of the thyroid gland. This finding suggests that this hormone produces an elevation of fibrinogen in rats by an extrathyroideal mechanism.

The mechanisms that modify the normal levels of plasma fibrinogen are not well known, although it recently has been demonstrated that both the hypothalamus and the hypophysis, and some of its hormones or products, are involved[2-7]. Besides, the hypothalamus-hypophysis-thyroid axis is very important in the regulation of plasma proteins[8,9]. In a previous study, we have found that the removal of the thyroid gland in rats increments plasma fibrinogen levels (unpublished data). Extirpation of thyroid gland leads to a decrease of the negative feedback exerted by thyroid hormones on the hypophysis and hypothalamus, with a consequent increment in thyroid stimulating hormone (TSH) levels. Thus, we thought it of interest to study the role of TSH on the levels of plasma fibrinogen.

Material and methods. 177 female rats weighing from 170 to 220 g and fed with a balanced diet (20% minimum of proteins) were used. Bovine TSH, NHI-TSH B6 (National Institute of Health, relative mean potency: 2.54 U.S.P. units/mg), highly purified, was used in doses of 12 µg/day. L-thyroxine (T4) and 3'3'5-triiodothyronine (T3) Sigma were employed in doses of 10 µg/day each one. The hormones were dissolved in alkaline saline solution[10] and administrated by daily i.m. injection. Propylthiouracil (PTU) (Gramón) was given in 0.05% solution in drinking water. 10 days after the beginning of the treatment or surgical operation, blood was extracted by decapitation. A mixture of potassium oxalate in 2:1 proportion was used to avoid blood coagulation. The concentration of fibrinogen was determined by RATNOFF et al.[11] method. Values of fibrinogen of 14 healthy intact rats were considered as normal controls. Thyroidectomy was performed through neck incision[12]. Both the thyroid and parathyroid glands were removed at the operation.

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However, the term 'thyroidectomized' will be used to describe these animals. After the operation, the rats were given 1% calcium chloride in their drinking water. Sham thyroidectomy involved all the steps of thyroidectomy, including manipulation of the thyroid gland, but stopped before its removal. All the operations were performed under ether anesthesia. Red cell volume was determined by the Wintrobe hematocrit.

The following groups of rats were studied: 1. normal intact rats: a) without treatment (control); b) injected with: TSH; T4; T3; and solvent. 2. Thyroidectomized rats: a) without treatment (Tx); b) injected with: TSH (TxTSH); T4 (TxT4); T3 (TxT3); TSH and T4 (Tx TSHT4); TSH and T3 (TxTSHT3); c) sham thyroidectomized rats. 3. Rats with propylthiouracil (PTU): a) without treatment; b) injected with TSH. The Student's *t*-test was used for the statistical analysis.

Table I. Plasma fibrinogen levels in normal intact rats and in rats injected with TSH, T3, T4 or solvent

	No. of animals	Fibrinogen (mg/100 ml)	*P*
Normal intacts rats (control)	13	245.6±10.3[a]	
TSH	23	402.5±11.9	<0.001
T4	7	222.4±14.2	
T3	10	206.2±20.4	
Solvent	9	218.5±22.7	

[a] Mean ± SE *P* = degree of significance resulting from comparison of the other groups with control. *P*-values are shown only when the difference is significant.

Table II. Effects of administration of TSH, T3 or T4 on plasma fibrinogen levels in thyroidectomized rats

	No. of animals	Fibrinogen (mg/100 ml)	*P*
Tx (control)	12	322.7±15.6[a]	
TxTSH	19	408.2±15.7	<0.001
TxT4	11	224.0± 7.1	<0.001
TxT3	8	209.3±19.0	<0.001
TxTSHT4	16	393.5±15.8	<0.01
TxTSHT3	10	380.9±13.6	<0.02
Sham operated	11	236.6±23.2	<0.01

[a] Mean ± SE. *P* = degree of significance resulting from comparison of the other groups with control. *P*-values are shown only when the difference is significant.

Table III. Effects of TSH administration on plasma fibrinogen levels in rats after propylthiouracil treatment

	No. of animals	Fibrinogen (mg/100 ml)	*P*
Propylthiouracil (control)	9	310.2±26.5[a]	
Propylthiouracil + TSH	19	409.5±17.0	<0.01

[a] Mean + SE. *P* = degree of significance resulting from comparison of the other groups with control. *P*-values are shown only when the difference is significant.

Results. The values of fibrinogen obtained in normal rats, and in those injected with solvent, T3 or T4, are presented in Table I. The normal values were similar to those found by other authours using different methods[13,14]. Administration of TSH increased the fibrinogen significantly in normal rats. Solvent itself did not modify fibrinogen. On the other hand, administration of T3 or T4 to normal rats did not lead to an increment of fibrinogen such as that observed after TSH administration.

The results obtained in rats submitted to surgical thyroidectomy are presented in Table II. In TxTSH rats fibrinogen levels increased significantly in Tx animals, whereas no significant differences were found when comparing TxTSH rats with normal rats injected with TSH. Thyroidectomy itself increased fibrinogen levels, whereas sham operation did not. On the contrary, in either TxT4 or TxT3 rats the concentration of fibrinogen decreased significantly. On the other hand, in TxTSHT4 or TxTSHT3 groups, fibrinogen increased significantly only in comparison with either TxT4 or TxT3 groups, but not with respect to normal rats injected with TSH.

In normal rats the hematocrit was 44.2 ± 0.98, and no significative differences were found with the other groups.

As shown in Table III, chemical thyroidectomy with PTU treatment[15] incremented fibrinogen to values similar to those obtained in Tx rats (Table II). On the other hand, after administration of TSH, the increment was similar to that obtained in TxTSH rats and normal rats injected with TSH.

Discussion. The finding that TSH produces elevation of the plasma fibrinogen levels of the same magnitude in normal, operative or chemically thyroidectomized rats, indicates that its action is exerted through an extrathyroideal mechanism.

Inasmuch as in the sham operated group fibrinogen values remained unchanged, it is discarded that the elevation observed after surgical thyroidectomy may be caused by tissue injury[16] or tissue inflammation[13] conditions in which fibrinogen increases shortly after injury.

The elevation of fibrinogen found in Tx animals could be accounted for by the higher endogenous levels of TSH. On the other hand, the decrease of fibrinogen in either TxT4 or TxT3 groups could be explained by the negative feed back exerted by T4 or T3 on TSH secretion[17–19]. Since T4 does not modify fibrinogen synthesis in the rat liver[20], it can be discarded that the decrease in fibrinogen after T4 administration in Tx animals is due to such an effect.

Neither T4 nor T3 decreased fibrinogen when administrated together with exogenous TSH. Under these conditions, TSH is not under control of a negative feedback mechanism. PTU treatment excludes the possibility that extirpation of the parathyroid glands may be the cause of the increment in fibrinogen observed after operative thyroidectomy. The variations of fibrinogen are also unrelated to alterations in red cell-plasma ratio.

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