neic effect for the induction of antibody are critical since an exagerated or prolonged allogeneic stimulation results in suppression of antibody formation[14]. Since low-dose irradiation increases the allogeneic effect[15], this could explain the absence of thyroglobulin antibodies in X-irradiated mice. Furthermore, low-dose irradiation inhibits suppressor activity[21] and separate suppressor mechanisms for the thyroid lesions and thyroglobulin antibody were described during the induction of autoimmune thyroiditis[22]. The allogeneic effect obtained after irradiation could induce high-affinity thyroglobulin antibodies which are readily fixed by the thyroid and not found in the circulation. They could trigger the induction of thyroid infiltrates. Such a mechanism cannot be firmly proven since the half-life of mouse IgG is only 1.9 days and fluorescent studies on thyroid tissue are not helpful in detecting thyroglobulin antibodies in mice. In any event, the low-dose irradiation per se did not seem to increase the susceptibility of the thyroid to lymphocytic infiltration[7] since control irradiated mice did not show any thyroid damage as late as 22 days after irradiation.

These experiments seem to support the view that normal mice have thyroglobulin-reactive B lymphocytes[9-11]. A graft-versus-host reaction as can develop in humans after bone marrow transplants might stimulate an autoimmune reaction by acting as adjuvant for various autoantigens.

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Effects of epinephrine on plasma fibrinogen levels in rats submitted to tissue injury

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Summary. Tissue injury (laparotomy) produces an increase in plasma fibrinogen. This increase is inhibited by the removal of the adrenal medulla, but injection of epinephrine in laparotomized-medullectomized rats returns fibrinogen levels to values similar to those observed in only laparotomized rats. Epinephrine administration to laparotomized rats increases the fibrinogen compared with the group of laparotomized rats without treatment, but epinephrine by itself does not modify plasma fibrinogen levels in uninjured rats. Epinephrine is apparently responsible for the increase of plasma fibrinogen in rats subjected to tissue injury, probably through beta adrenergic stimulation.

In previous work it has been found that tissue injury increases plasma fibrinogen levels in rats[1-4]. ACTH increases the fibrinogen due to an extra-adrenal mechanism[5] and to an increase of hepatic fibrinogen synthesis[6,7]. On the contrary, adrenalectomy decreases plasma fibrinogen level[8], and corticosterone, which is main glucocorticoid hormone in rats, does not have any influence on it[9]. In the other hand, tissue injury is a noxious stimulus which activates both the sympatoadrenal medullary system and epinephrine secretion; and as the presence of adrenal epinephrine is necessary to produce an increase of alpha globulin in rats submitted to stress[10-13], we have studied, on the basis of these considerations, the role that epinephrine would have on plasma fibrinogen levels in rats submitted to tissue injury.

Material and methods. 133 rats of both sexes, Suquía Strain, weighing 140-220 g were used. They were fed with balanced food containing sufficient protein (20%). The animals were divided into 2 groups: Group I, rats with tissue injury (laparotomy); and group II, rats injected with adrenergic receptor blockers. Each of them included several subgroups. Group I: a) Laparotomized rats without other treatment (L); b) with extirpation of adrenal medulla (LMx); c) with extirpation of adrenal medulla and injected with epinephrine (LMxEp); d) laparotomized rats injected with epinephrine (LEp); e) adrenalectomized rats (LAx); f) normal intact rats: 1. without treatment (control); 2. injected with saline; 3. only with ether anesthesia; 4. injected only with epinephrine. Group II: a) Rats with laparotomy and injection of propranolol (LPr); b) with laparotomy and injection of phenoxybenzamine; c) laparotomized + propranolol + epinephrine (LPrEp); d) laparotomized + phenoxybenzamine + epinephrine (LPhEp); e) normal rats injected with propranolol (Pr) or with phenoxybenzamine.

Tissue injury was made by posterior laparotomy. It included careful manipulation of kidney and adrenal glands. Laparotomy was made with the rats in the prone

position, and it consisted of a dorsal midline incision about 2 cm long covering from nearly the tenth thoracic vertebra to the third lumbar one. Adrenal glands or adrenal medullas were removed from this central fur incision.

The peritoneal cavity and the fur were closed with silk sutures. Operations were made under ether anesthesia through inhalation. Normal uninjured rats were used as control. The drugs were dissolved in saline, and injected by the s.c. route twice a day. Epinephrine was administered at doses of 0.2 mg/kg/day[14], propranolol und phenoxybenzamine in doses of 2 mg/kg/day[14,15]. In laparotomized animals the administration of adrenergic blockers began 2 h before operation.

96 h after surgical operation or after beginning with drug administration, rats were bled by decapitation and blood was immediately transferred to centrifuge tubes containing 15 mg of a dry mixture of ammonium oxalate and potassium oxalate in a proportion of 2:1. Fibrinogen was determined by the Ratnoff and Menzie method[16]. In adrenalectomized rats, fibrinogen values were corrected according to the normal values of hematocrit, using the Boas and Peterman equation[17].

Student's t-test was used for statistical treatment. Significant differences were taken as $p < 0.05$.

Results. Results obtained after studying groups I and II are presented in the table. There are no significant differences between plasma fibrinogen levels when comparing the group of animals injected with saline and the group of normal intact rats without treatment. Laparotomy produces by itself a significant increase of plasma fibrinogen compared with normal intact controls ($p < 0.001$). Ether anesthesia itself does not modify plasma fibrinogen levels compared with normal control rats. On the other hand, in both LMx and LAx lots, plasma fibrinogen level is significantly less increased than that observed in the L group. On the contrary, in the group of LMxEp animals, fibrinogen increased significantly compared with the group of laparotomized rats without treatment ($p < 0.05$). The injection of epinephrine in intact rats did not modify the plasma fibrinogen levels compared with normal rats without treatment or with the lot of rats injected only with saline, but the fibrinogen increased significantly in the lot of LEp rats, compared with the lot of laparotomized rats ($p < 0.02$).

In order to verify whether the possible action of epinephrine on the increase of fibrinogen consecutive to the tissue injury was due to an action on either alpha or beta adrenergic receptors, or both of them, we studied rats injected with propranolol or phenoxybenzamine. In the laparotomized rats injected with propranolol (LPr), fibrinogen values were not modified significantly compared with normal intact rats or with (LPrEp) group. On the contrary the difference is significant if the lot of LPr rats is compared with the groups of both laparotomized uninjured rats and laparotomized rats injected with epinephrine (LEp). On the other hand, there are no significant differences when comparing the LPrEp lot with LAx or LMx groups.

In laparotomized animals injected with phenoxybenzamine or in LPhEp group, fibrinogen increases to values similar to those observed in laparotomized rats without treatment (L). The administration of either propranolol or phenoxybenzamine in normal uninjured rats did not influence plasma fibrinogen levels compared with normal control rats.

Discussion. The results obtained confirm that tissue injury produces significant increase of plasma fibrinogen. The following observations make us think that epinephrine participates in the increase of plasma fibrinogen in rats with tissue injury: a) Fibrinogen decreases in LMx rats compared with L group. b) There are no significant differences when comparing L and LMxEp groups. c) In the LEp group the increase of plasma fibrinogen is greater than in laparotomized rats. As in the LMx group fibrinogen decreases to values similar to those observed in LAx, and as corticosterone and mineralocorticoids do not modify plasma fibrinogen in rats[9], we think that the decrease of fibrinogen in adrenalectomized rats is owing to the absence of adrenal medulla and epinephrine.

ACTH increases the fibrinogen only through an extra-adrenal mechanism[5]. But as the presence of adrenal epinephrine and the integrity of the nervous hypothalamus-spinal cord-adrenal medulla pathway is necessary to increase some plasma proteins (albumin, alpha globulin) in rats[10–13], we think that tissue injury probably produces elevation of plasma fibrinogen through nervous adrenal medullary secretion.

The fact that the increment of plasma fibrinogen in tissue injury was blocked by propranolol and not with phenoxybenzamine (at the doses used) suggests to us that the action of epinephrine on plasma fibrinogen is a beta adrenergic effect, which must take place in the liver where fibrinogen is synthesized. On the other hand, phenoxybenzamine abolishes the effects of injected epinephrine in laparotomized rats but not of the laparotomy itself. This fact may be explained because phenoxybenzamine administered in rats injected with pharmacological doses of

Effects of adrenal medullectomy, adrenalectomy, epinephrine, propranolol, phenoxybenzamine, ether anesthesia and saline on plasma fibrinogen levels in rats with tissue injury

	Number	Fibrinogen (mg/100 ml)	p
Intact rats (control)	9	225.8 ± 12.6*	
Saline	7	235.0 ± 14.2	
Ether anesthesia	7	248.2 ± 21.8	
Laparotomized rats (L)	9	335.2 ± 15.9	< 0.001
Laparotomized + medullectomized (LMx)	10	285.3 ± 15.4	< 0.01
Laparotomized + medullectomized + epinephrine (LMxEp)	8	378.2 ± 18.6	< 0.001
Laparotomized + adrenalectomized (LAx)	13	268.3 ± 16.2	< 0.05
Laparotomized + epinephrine (LEp)	9	392.0 ± 13.2	< 0.001
Epinephrine	8	230.0 ± 14.2	
Laparotomized + propranolol (LPr)	9	268.0 ± 20.8	
Laparotomized + propranolol + epinephrine (LPrEp)	8	272.2 ± 20.0	
Propranolol (Pr)	9	237.4 ± 17.3	
Laparotomized + phenoxybenzamine	9	318.9 ± 24.8	< 0.001
Laparotomized + phenoxybenzamine + epinephrine	10	300.1 ± 24.1	< 0.02
Phenoxybenzamine	8	230.0 ± 14.2	

* Mean ± SE. p is indicated when the differences were significant.

epinephrine produces an additional beta adrenergic blockade[9]. Research now in progress shows that in laparotomized rats injected with epinephrine and phentolamine (another alpha adrenergic blocker), plasma fibrinogen levels increase to values similar to those observed in the LEp group.

The absence of alteration in the fibrinogen level in normal intact rats injected with propranolol would indicate that an eventual decrease of epinephrine binding to hepatic cell receptors does not influence the plasma fibrinogen levels when tissue injury is not performed. On the contrary, it confirms that epinephrine effects on fibrinogen are only evidenced in rats submitted to tissue injury.

The beta adrenergic effect of epinephrine may be indirect, through its action on the synthesis of TSH or insulin hormones[18-20]. These hormones contribute to the increase of plasma fibrinogen in tissue injury[3,4]. In conclusion, according to our results, epinephrine would be responsible for the increase of plasma fibrinogen level in laparotomized rats.

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Stimulation of the incorporation of ^{3}H-leucine into proteins by oestradiol in the foetal uterus of the guinea-pig

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Summary. Oestradiol treatment increases the incorporation of ^{3}H-leucine into acid insoluble proteins in the foetal guinea-pig uterus (50–65 days of gestation) 10 times above control values by 8 h and 20 times by 24 h after administration of oestradiol to the mother.

The foetal guinea-pig uterus has been shown to respond to oestrogen treatment by an increase in wet weight[1] and a great stimulation of the progesterone receptor protein[2]. Moreover, these responses can be correlated with the translocation and nuclear retention of oestrogen receptor in the foetal uterus[3]. The present study now demonstrates that oestradiol also has an effect on the in vivo incorporation of ^{3}H-leucine into acid-insoluble proteins in the foetal uterus.

Pregnant guinea-pigs of the Hartley albino strain were obtained from a commercial breeder (Centre d'Elevage R. Janvier, Le Genest, France) and varied from 50 to 65 days of gestation. These animals were injected s.c. with 1 mg oestradiol/kg b.wt in 50% ethanol-saline (controls were given vehicle alone). 8 h or 24 h later, the animals were anesthetized with ether, the foetuses were exposed by laparotomy and each female foetus was injected with 60 µCi (1.6 µg or 0.012 µmoles) of ^{3}H-leucine (sp.act. 5 Ci/mmole, New England Nuclear, Dreieich, FRG) in 0.25 ml 0.1 N HCl. The foetuses were replaced in the abdomen and 30 min later the animals were sacrificed and the foetal uteri excised. Each uterus was homogenized in 1 ml cold distilled water and 1 ml of 1 N perchloric acid (PCA) was added. After 15 min, the homogenate was centrifuged at 900 × g for 10 min and the acid-insoluble precipitate was washed 4 times with 2 ml cold 0.2 N PCA. The washed precipitate was resuspended in 2 ml 0.5 N PCA, hydrolyzed at 90 °C for 30 min and centrifuged to obtain a supernatant which was assayed for DNA by the method of Burton[4] and a final precipitate which was completely dissolved in 2 ml of 0.1 N NaOH by heating at 50 °C for 30 min. Protein concentration was measured as described by Lowry et al.[5] and an aliquot was counted (46% efficiency) in Ready-Solv HP (Beckman Instruments, Gagny, France).

The figure shows that oestradiol treatment increases the in vivo incorporation of ^{3}H-leucine into acid-insoluble protein 10 times above control values in the non-treated animals by 8 h after treatment and 20 times by 24 h ($p < 0.001$ between the 8-h and 24-h values). Thus, the foetal guinea-pig uterus

Effect of oestradiol treatment on total acid-insoluble protein and total DNA in foetal guinea-pig uterus

	mg protein/ g tissue	mg DNA/ g tissue	mg protein/ mg DNA
Untreated control	75.22 ± 5.11	5.47 ± 0.83	15.11 ± 1.80
8-h oestradiol	61.21 ± 2.00	4.89 ± 0.75	13.47 ± 3.00
24-h oestradiol	66.05 ± 4.59	4.45 ± 0.50	15.97 ± 3.20

Protein and DNA were determined in the samples described in the figure. The values represent the means ± SEM.