

## EFFECTS OF THYROIDECTOMY AND L-THYROXINE ON ADRENALINE AND NORADRENALINE CONCENTRATIONS IN THE ADRENAL GLANDS AND PLASMA OF RATS DURING THE PRO-OESTROUS PHASE OF THE OESTROUS CYCLE AND PREGNANCY

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- 1 The influence of thyroidectomy upon the adrenaline and noradrenaline content of adrenal glands and plasma in mature female rats in pro-oestrus and in pregnant rats was studied.
- 2 Adrenal adrenaline and noradrenaline declined significantly after thyroidectomy in pro-oestrous and pregnant females but the effects were more marked in pregnant females.
- 3 Plasma adrenaline increased by 160% after thyroidectomy in pro-oestrous females but similar treatment resulted in 85% decrease in plasma adrenaline of pregnant rats. The loss of thyroid increased plasma noradrenaline significantly in both groups of females.
- 4 The administration of L-thyroxine to thyroidectomized females increased adrenal noradrenaline stores of both the groups. The effects of L-thyroxine in pro-oestrous females resulted in decreased adrenaline stores of the adrenals but the pregnant group showed an increase. Plasma noradrenaline increased after treatment of pro-oestrous and pregnant-thyroidectomized females with L-thyroxine.
- 5 The thyroidectomized females in pro-oestrous phase receiving L-thyroxine showed a return to the control values for plasma adrenaline but in pregnant females whose plasma adrenaline had declined after thyroidectomy no such change occurred.
- 6 Considering the variations in total catecholamines in plasma and adrenals, it was observed that the loss of thyroid hormones results in an increase in total catecholamine storage and output in the blood. The results provide evidence that the thyroid-catecholamine interrelation is significantly affected by pregnancy.

### Introduction

The influence of endocrine gland secretions upon the processes of monoamine synthesis, release and metabolism has been a subject of interest during the past decade (Axelrod, Mueller & Thoenen, 1970; von Euler, 1972). More recently the biochemical mechanisms that may stimulate or inhibit the synthesis or breakdown of the monoamines under the influence of different hormones have been examined at the molecular level (Wurtman, Pohorecky & Baliga, 1972). The fundamental work on the effects of thyroid hormones on regulation of monoamines was provided by Wurtman, Kopin & Axelrod (1963) and Landsberg & Axelrod (1968), who analysed the relationship

between thyroid function and the cardiac disposition of catecholamines. The latter authors found that thyroid hormones can influence both monoamine oxidase and catechol-O-methyltransferase since a few minutes after a dose of adrenaline, the amount found in the hyperthyroid rat heart was considerably smaller than in the normal heart. A study of the tritiated metabolites formed from tritiated adrenaline *in vivo* on the hyperthyroid rat heart showed a decrease in the cardiac content of all the main metabolites, while they were increased in hypothyroidism (Landsberg & Axelrod, 1968). Most of these studies have been confined to normal animals and little attention has been given to the effect of hormones on monoamine regulation during hormonal deficiencies. Recently, evidence has been provided that the effects of drugs

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and hormones on monoamine metabolism are greatly modified during various physiological states such as pregnancy and the oestrous cycle (Parvez, Parvez & Youdim, 1975; Parvez & Parvez, 1976; Parvez, Parvez, Raza-Bukhari & Youdim, 1976). The present experiments were performed to study the influence of thyroidectomy during pregnancy and in pro-oestrus upon the storage and release of adrenaline and noradrenaline from adrenal gland into plasma. Thyroidectomized animals were also given L-thyroxine to examine the effects of this hormone on the two parameters studied.

## Methods

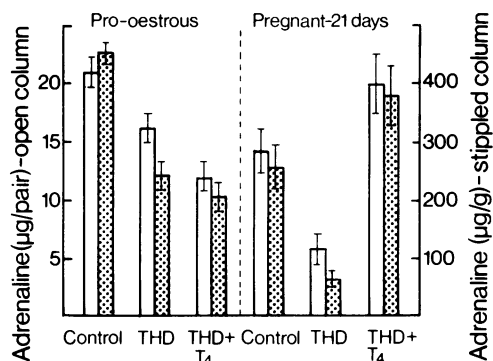
Albino female rats of Sherman strain obtained from Janvier, Paris were used in all experiments. The females weighed  $250 \pm 50$  g (range) and were housed at a constant temperature of  $21^\circ\text{C}$  with natural day and night cycles. They were fed with commercial laboratory food *ad libitum*. The females were impregnated by keeping one male in the cage of seven females from 18 h 00 min to 09 h 00 min only once. The fertilization was assumed to occur at 02 h 00 min. Thus, it was possible to make an estimation for an approximation of pregnancy  $\pm 7$  hours. The females were palpated abdominally 14 days later for the verification of pregnancy. Under normal conditions parturition took place during Days 21.5 and 22 of gestation (Parvez, Grippo & Parvez, 1974).

### Thyroidectomy and L-thyroxine administration

The animals were thyroidectomized on Day 15 of pregnancy or during the well defined pro-oestrous phase of the oestrous cycle. The operation was performed under ether anaesthesia according to the technique of Zarrow, Yochim, McCarthy & Sanborn (1964). All the operated rats were examined for the effectiveness of thyroidectomy by measuring their weights 3 days after the operation. The administration of L-thyroxine (Nutritional Biochemicals) started on the day after operation and lasted for five days. All the thyroidectomized rats received  $3 \mu\text{g}/100$  g body weight of L-thyroxine daily at 10 h 00 min. On the sixth day, the animals were killed by neck fracture and the blood taken and adrenals excised in less than 1 minute. A maximum of 3 ml of blood was taken from the abdominal aorta of each rat.

### Assay of adrenaline and noradrenaline in plasma

Heparinized blood from 5 rats was pooled in a glass centrifuge tube containing 2 ml of potassium fluoride (2%) and 2 ml of potassium thiosulphate (3%). The blood was centrifuged at  $1^\circ\text{C}$  for 10 min at 10,000 rev/minute. Adrenaline and noradrenaline in



**Figure 1** The influence of thyroidectomy and L-thyroxine administration (s.c.) upon adrenaline storage in adrenal gland of pregnant rats and rats during pro-oestrous phase of the oestrous cycle. The results are expressed in  $\mu\text{g}/\text{pair}$  (open columns) as well as  $\mu\text{g}/\text{g}$  (stippled columns) of adrenals. Control (non-operated rats on 15th day of pregnancy or during pro-oestrus), THD (thyroidectomized) and THD + T<sub>4</sub> (thyroidectomized rats receiving L-thyroxine s.c.). Each group contained 8 to 16 rats and each column represents mean result, vertical lines show s.e. mean.

Significance of differences between groups:

	Pro-oestrus	Pregnant
Control vs THD	$P < 0.01$ ( $\mu\text{g}/\text{pair}$ ) $P < 0.001$ ( $\mu\text{g}/\text{g}$ )	$P < 0.001$ ( $\mu\text{g}/\text{pair}$ ) $P < 0.001$ ( $\mu\text{g}/\text{g}$ )
THD vs THD + T <sub>4</sub>	$P < 0.02$ ( $\mu\text{g}/\text{pair}$ )	$P < 0.001$ ( $\mu\text{g}/\text{pair}$ ) $P < 0.001$ ( $\mu\text{g}/\text{g}$ )

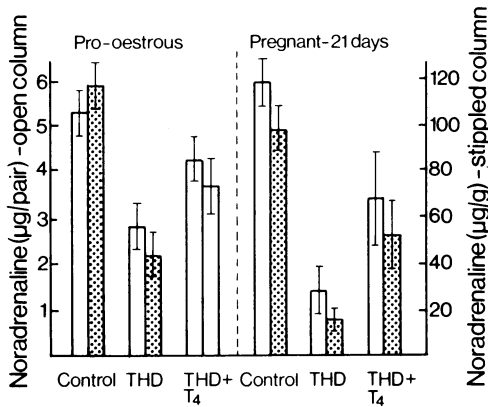
plasma were isolated by adsorption on acid activated aluminium oxide (von Euler & Lishajko, 1959). The batch process was preferred to columns since the recoveries were better (Anton & Sayre, 1962). One g of alumina was washed twice with quartz distilled water and added to the 2 ml of plasma in a centrifuge tube. The pH was adjusted to 8.4 and the adsorption allowed to continue with gentle agitation for 4 minutes. The tubes were then centrifuged at 3000 g for 1.5 min and the supernatant discarded. The alumina was washed with 20 ml of distilled water and the catecholamines eluted with 4 ml of 0.25 N acetic acid. The trihydroxyindole procedure was employed for simultaneous estimation of both adrenaline and noradrenaline.

The mean values are given together with the standard errors of the means, and statistical comparison made using Student's *t* test.

## Results

### Effect of thyroidectomy on adrenal gland adrenaline

Figure 1 illustrates the influence of thyroidectomy and compensatory treatment with L-thyroxine upon



**Figure 2** Noradrenaline storage in adrenals of females thyroidectomized during pro-oestrous phase or on 15th day of pregnancy. THD=thyroidectomized rats, T<sub>4</sub>=-thyroxine given subcutaneously. Each group represents mean of 8 to 16 values; vertical lines show s.e. mean.

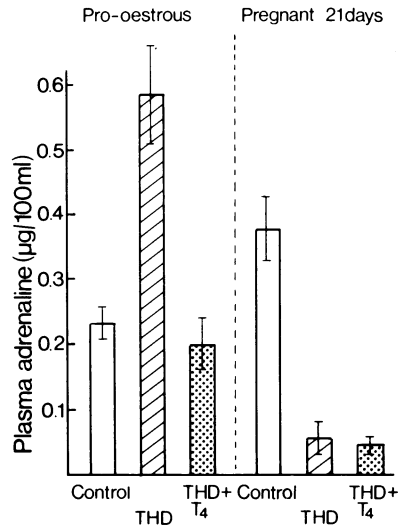
Significance of differences between groups:

	Pro-oestrous	Pregnant
Control vs THD	$P < 0.001$ (µg/pair)	$P < 0.001$ (µg/pair)
	$P < 0.001$ (µg/g)	$P < 0.001$ (µg/g)
THD vs THD + T <sub>4</sub>	$P < 0.01$ (µg/pair)	$P < 0.02$ (µg/pair)
	$P < 0.01$ (µg/g)	$P < 0.02$ (µg/g)

adrenaline storage in the adrenal gland of female rats during the pro-oestrous phase of the oestrous cycle and in late pregnancy. The results have been expressed in µg/pair of adrenal glands as well as in µg/g of adrenal weight. The adrenaline content of the adrenal gland in the pregnant rats was lower than in the controls ( $P < 0.01$ ), confirming earlier results (Parvez *et al.*, 1976) obtained with rabbits. The removal of the thyroid gland resulted in a significant decrease in the adrenaline content of the adrenal glands in both groups of females. The decrease was 32% (µg/pair) and 55% (µg/g) for adrenals of the rats in pro-oestrous, and in the pregnant group it was 63% (µg/pair) and 74% (µg/gram). Compensatory treatment of pro-oestrous females with L-thyroxine further reduced the adrenaline stores in the adrenal gland but the pregnant females responded differently to this treatment by showing an increase, 355% (µg/pair) and 567% (µg/gram). The mean values in thyroidectomized females receiving L-thyroxine during pregnancy were close to the control values.

#### *Effect of thyroidectomy on adrenal gland noradrenaline*

The storage of noradrenaline in the adrenal gland of female rats thyroidectomized during the pro-oestrous phase of the oestrous cycle and on 15th day of pregnancy is shown in Figure 2. Thyroidectomy



**Figure 3** Variations in plasma adrenaline after thyroidectomy and compensatory treatment of L-thyroxine to cyclic or pregnant rats. Each group represents mean of 16 to 18 females; vertical lines show s.e. mean. THD=thyroidectomized, and THD+T<sub>4</sub>=thyroidectomized rats receiving L-thyroxine (T<sub>4</sub>).

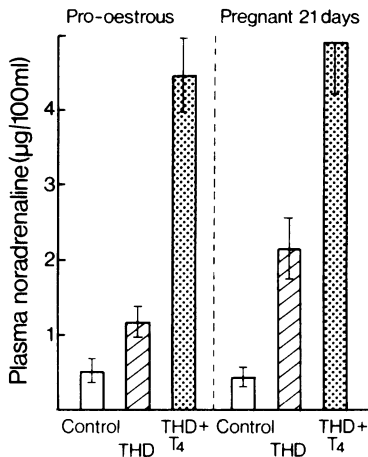
Significance of difference between the groups:

	Pro-oestrous	Pregnant
Control vs THD	$P < 0.001$	$P < 0.001$
THD vs THD + T <sub>4</sub>	$P < 0.001$	NS
Control vs THD + T <sub>4</sub>	NS	$P < 0.001$

resulted in a decrease of 47% (µg/pair) and 63% (µg/g) when compared with values of control female rats in the pro-oestrous phase, and in a 77% and an 84% decrease (µg/pair and µg/g respectively) in the adrenal noradrenaline content of the pregnant group when compared with non-operated pregnant rats. The administration of L-thyroxine to thyroidectomized females in both groups of rats resulted in an increased storage of noradrenaline and the mean values showed a tendency towards the non-operated values. The extent of noradrenaline increase in pregnant females was greater than in pro-oestrous females.

#### *Effect of exogenous thyroxine on the plasma concentrations of adrenaline and noradrenaline*

The variations in plasma concentrations of adrenaline after removal of the thyroid gland in female rats during pro-oestrous and on Day 15 of pregnancy are shown in Figure 3. After thyroidectomy the adrenaline content of plasma in pro-oestrous females increased by 160%. The removal of the thyroid gland in the pregnant rats produced an opposite effect; the adrenaline content of plasma fell by 85%. The treatment of thyroidectomized pro-oestrous rats with



**Figure 4** Plasma noradrenaline content in thyroidectomized female rats operated during oestrous cycle or on day 15 of pregnancy. THD=thyroidectomized; T<sub>4</sub> = L-thyroxine treated. Each group is mean value from 18 rats; vertical lines show s.e. mean.

Significance of difference between the groups:

	Pro-oestrus	Pregnant
Control vs THD	$P < 0.01$	$P < 0.001$
THD vs THD + T <sub>4</sub>	$P < 0.001$	$P < 0.001$

L-thyroxine lowered the adrenaline content of plasma but the mean value was not significantly different from that of controls. L-Thyroxine administration to pregnant thyroidectomized rats did not produce any change in plasma adrenaline and the mean value remained significantly lower than in the non-operated pregnant rats.

Figure 4 provides a comparison of plasma noradrenaline in pro-oestrous and pregnant rats after thyroidectomy and compensatory treatment with L-thyroxine. The removal of the thyroid gland resulted in 125% increase in plasma noradrenaline of females during the pro-oestrous phase of the oestrous cycle. The effect of thyroidectomy in pregnant females was similar to that in pro-oestrous females but the increase in noradrenaline content of plasma was much higher (393% higher than control value). The administration of L-thyroxine to rats in pro-oestrous phase resulted in a marked increase in plasma noradrenaline content. Similar treatment of pregnant thyroidectomized females with L-thyroxine also produced a significant increase in the concentration of the transmitter in the plasma.

The variations in total catecholamine (adrenaline + noradrenaline) content of plasma and adrenal gland after thyroidectomy as well as after compensatory treatment with L-thyroxine in pregnant and pro-oestrous animals are shown in Table 1. The per cent changes from non-operated controls in both the

groups are also indicated. These parameters have been calculated to show that the effects of thyroid deficiency during normal life and pregnancy differ to a great extent for the storage of catecholamines in the adrenal medulla. The changes in total plasma amine levels which have been observed suggest that each catecholamine changes greatly without modifying the total catecholamine concentration. The response to L-thyroxine administration in thyroidectomized females during pregnancy was always greater than that in rats in the pro-oestrous phase of the oestrous cycle. These results show that hormonal sensitivity changes considerably during pregnancy for the processes of monoamine storage and release.

## Discussion

In addition to many other well known effects upon endocrine metabolism, removal of the thyroid gland influences the storage and release of adrenaline and noradrenaline from the adrenal gland during the pro-oestrous phase of the oestrous cycle as well as during pregnancy. The present results show that the noradrenaline content of the adrenal gland declines after thyroidectomy in both pro-oestrous and pregnant animals by 47% and 84% respectively, whereas at the same time a significant increase in plasma noradrenaline concentration in both the groups of animals occurs which would suggest that there is an accelerated release of noradrenaline from the adrenal gland into the circulation. Previous study of the role of thyroid hormones on noradrenaline turnover and metabolism in the rat heart suggests that thyroid deficiency is associated with a significant decrease in endogenous noradrenaline content of the heart, possibly mediated by modifications in sympathetic nervous activity (Landsberg & Axelrod, 1968). During hypothyroidism in the rat the activity of adrenal gland monoamine oxidase is only 55% of the normal value in the male rat but in the female there is no change (Wurtman *et al.*, 1963). This observation leads to the suggestion that intracellular degradation of monoamines affected by monoamine oxidase remains unchanged in the hypothyroid female rat and the marked increase in plasma noradrenaline is mainly caused by accelerated release from the adrenal gland. Moreover it was also found that the activity of enzyme catechol-O-methyltransferase was unaffected by removal of the thyroid gland and these effects were similar in male and female rats (Wurtman *et al.*, 1963). This evidence is in agreement with the present results since the rate of degradation of plasma noradrenaline remained at a steady state after thyroidectomy and the increased release from the adrenal gland can be detected in the blood.

Adrenaline in the adrenal glands of pro-oestrous and pregnant females reacted in a similar manner to

**Table 1** Variations in total catecholamines (adrenaline (Ad) + noradrenaline (NA)) in adrenal gland and plasma of female rats thyroidectomized during pro-oestrous phase of the oestrus cycle or on the day 15 of pregnancy. The effects of L-thyroxine ( $T_4$ ) administered for 5 days ( $3 \mu\text{g}/100 \text{ g}$  body weight daily) are also shown.

Treatment	Adrenal Ad + NA ( $\mu\text{g}/\text{pair}$ )	% of non-operated controls	Adrenal Ad + NA ( $\mu\text{g}/\text{g}$ )	% of non-operated controls	Number of cases	Plasma Ad + NA ( $\mu\text{g}/100 \text{ ml}$ )	% of non-operated controls	Number of cases
Thyroidectomized in pro-oestrus	$19 \pm 1.7$	73	$298 \pm 33.5$	52	10	$1.75 \pm 0.28$	233	16
Thyroidectomized in pregnancy	$7.1 \pm 1.84$	34	$83 \pm 20.7$	23	13	$2.2 \pm 0.41$	275	18
Thyroidectomized in pro-oestrus + $T_4$	$16.2 \pm 1.7$	62	$280 \pm 35.4$	48	8	$4.6 \pm 0.5$	613	16
Thyroidectomized in pregnancy + $T_4$	$23.8 \pm 3.6$	112	$431 \pm 64$	120	8	$4.93 \pm 0.69$	616	16

The % values have been calculated from non-operated mean value of each respective group.

Thyroidectomized vs Thyroidectomized +  $T_4$

Significance of differences between the groups:

Pregnant  
Adrenals  $\mu\text{g}/\text{g}$  and  $\mu\text{g}/\text{pair}$  ( $P < 0.001$ )  
Plasma ( $P < 0.002$ )

Pro-oestrus  
Adrenals (NS)  
Plasma ( $P < 0.001$ )

its precursor noradrenaline since both groups showed a significant decline from normal values after thyroidectomy. The pro-oestrous females showed a significant increase in plasma adrenaline after thyroidectomy but the pregnant females had a lowered plasma adrenaline concentration after removal of the thyroid gland. This difference can be explained on the basis of modifications in endocrine status of pregnant females which is affected by the state of gestation. It is well established that metabolic effects mediated by the thyroid gland are greatly influenced during pregnancy (Furth & Pagliara, 1971). The thyroxine binding globulin capacity increases in pregnancy 2.5-fold (Robbins & Rall, 1967). Serum from pregnant animals also has a subnormal thyroxine binding pre-albumin concentration (Woeber & Ingbar, 1968). Suppression or augmentation of hormonal activity by thyroxine binding pre-albumin or thyroxine binding globulin is altered during pregnancy mainly as a result of reduction in chemical activity of steroid hormones (Westphall, 1970). The rise in thyroxine binding serum proteins during pregnancy has been recently suggested to be a consequence of oestrogen stimulation and this effect can also be seen in non-pregnant rats receiving oestrogen treatment (Furth & Pagliara, 1971). This rise in the binding capacity of protein results in an increased concentration of plasma thyroxine (Nikitovitch & Knobil, 1955). Because the binding capacity of serum protein is increased in pregnancy, the renin uptake is decreased (Sterling & Brenner, 1966). Considering evolutionary changes in the ratio of free and bound corticosteroids, oestrogens and progesterone, one can easily suggest that thyroid function which is more or less dependent on the normal function of the above hormones is certainly affected by the fluctuations which take place in these hormones during pregnancy (Wiest, Kidwell & Balough, 1968; Lau, Liao & Petropoulos, 1971; Shaikh, 1971). All these hormones have been shown to have a direct effect on the enzymes of monoamine metabolism (Phorecky & Wurtman, 1971; Parvez & Parvez, 1973; Holzbauer & Youdim, 1973; Parvez *et al.*, 1975; Parvez *et al.*, 1976). Therefore any consequent change in corticosteroids, oestrogens or progesterone possibly induced by thyroid deficiency should directly affect the plasma level of adrenaline in pregnant rats. Another piece of evidence which should also be

considered is that the percentage augmentation of noradrenaline in the adrenal glands is much greater in pregnant rats. Therefore very little adrenaline appears to be formed from the noradrenaline still present in the adrenals, and which can eventually be released from the gland. These modifications appear to be related to the differences observed between pregnant and cyclic animals. In pregnant rabbits the adrenaline content of adrenal gland declines throughout the gestational period and appears to be related to the fall in the activity of the enzyme phenylethanolamine-*N*-methyltransferase (Parvez *et al.*, 1976).

The effect of thyroidectomy upon noradrenaline storage in the adrenals and its plasma concentration was similar in pro-oestrous and pregnant rats. The increased plasma noradrenaline after removal of thyroid and administration of L-thyroxine confirms the previous observations of von Euler (1954) that adrenal secretion has very little influence on blood noradrenaline. It appears that deprivation of thyroid hormones stimulates sympathetic nerve terminals which liberate or synthesize more noradrenaline. It has been shown that monoamine synthesis in brain and peripheral neurones increases in hypothyroidism and decreases in hyperthyroidism (Prange, Meek & Lipton, 1970). The high levels of plasma noradrenaline observed in thyroidectomized rats after thyroxine treatment suggests that the dose employed was not large enough to restore the transmitter concentration to its normal levels. It can be seen (Table 1) that the total catecholamines in the adrenals were only slightly modified after the injections of thyroxine but plasma concentrations increased. This observation suggests an activation of adrenergic nerve terminals which can eventually release excessive amounts of noradrenaline.

In conclusion our results provide evidence that thyroid monoamine interrelations are modified to a great extent during pregnancy. The treatment of thyroid deficiency with L-thyroxine has a qualitatively different effect in the normal and pregnant animals.

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