control animals gained 45 g during the 11-day experimental period, tremorine-treated animals gained an average of 24 g. This effect on body weight was not reflected on brain weight. When brain weights were directly compared among the groups, they were not significantly different.

The lack of eating in the treated animals would be interpreted to reflect possible effects of tremorine on the hypothalamus although no direct supportive evidence is known. Another factor besides lack of eating which might be involved in the loss of weight could be the effect of the daily produced tremor, a stressful stimulus, on the adrenocortical system. An increased secretion of adrenocortical steroids due to stress would result in both catabolism and antianabolism of muscle protein which adrenocortical steroids are known to exert.

Several studies[2-4] have attempted to explain the tremor phenomena produced by tremorine and oxotremorine by increased levels of brain ACh. Moreover, attempts have been made to explain increased levels of ACh, either by decrease in activity of AChE, the hydrolysing enzyme of ACh, or increase in the activity of choline acetyltransferase, the synthesizing enzyme of ACh. The present marked increase of AChE in the cerebral cortex observed with the highest dose of tremorine (Table I) does not substantiate such a correlation. Also, recent studies by BARTOLINI et al.[3] have shown that ACh level is raised in the diencephalon-midbrain but not in the cerebral cortex after acute oxotremorine administration. Since in the present study no changes were observed in the AChE activity in the diencephalon-midbrain, the increased ACh levels observed by BARTOLINI et al.[3] cannot be explained by changes in the activity of the hydrolysing enzyme. It is suggested that the high AChE activity in the cerebral cortex after tremorine observed in this study may reflect a high turnover rate of ACh and thus explain the lack of changes in ACh level in this CNS structure.

Résumé. La trémorine a été administrée chez les rats quotidiennement pendant 7 jours à des doses de 7.5, 22.5 ou 30 mg/kg. Les témoins ont montré des mouvements convulsifs proportionnels à la dose de trémorine. Quatre jours après l'arrêt du traitement, l'activité acétylcholinestérasique du cortex cérébral était augmentée. Il est proposé que ce change de l'activité acétylcholinestérasique reflète une utilisation accrue de l'acétylcholine corticale.

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The Rate Limiting Control of Enzymes Monoamine Oxidase and Catechol-*O*-Methyl Transferase in the Foetus and the Adult by Adreno-Cortical Hormones

Early studies suggested that hormones of the adrenal cortex and catecholamines act as a single physiological unit[1,2]. The shifts in the concentration of corticosteroids greatly affect adrenaline and noradrenaline synthesis, release and urinary excretion[3-5]. Studies in the past decade have provided compelling evidence that adrenal cortical steroids play an essential role in the physiology of medullary chromaffin tissue[6,7]. The importance of glucocorticoids in noradrenaline methylation to adrenaline and the induction of enzyme phenylethanolamine-N-methyl transferase (PNMT) have been extensively investigated by WURTMAN and AXELROD[8-11]. Our recent observations indicate that there is a significant rise in output of catecholamine metabolites after hypophysectomy or adrenalectomy[12,13]. To confirm this hypothesis, rabbit foetuses were deprived of their hypophysis by decapitation in utero at the age of 20 days to inactivate the adrenal cortex[14,15]. The activities of enzymes monoamine oxidase (MAO) and catechol-*O*-methyl transferase (COMT) in adrenals, kidneys, paraganglia and lung of decapitated foetuses at the age of 31 days were measured. Adrenalectomy was also performed in new born rats at the age of 0 h and 10 days after their MAO activity was compared with that of normal young rats. To see if adreno-cortical hormones inhibited MAO and COMT in the adult rats, glucocorticoid synthesis was blocked with metopirone[16-18] and the activities of these two enzymes were determined 8 h after metopirone administration.

Materials and methods. White rabbits of New Zealand strain and Sherman rats were utilized throughout the experiments. The female rabbits were made pregnant in our labotarory and verified on the 14th day by palpation. The mothers were operated on the 20th day under nembutal anaesthesia and foetal hypophysectomy by decapitation was performed[14,15]. The maximum number of foetuses decapited from each mother ranged from 2 to 4. Another group of foetuses from unoperated mother also served as controls. 16 decapited foetuses in groups of 8 each were administered with 1.5 mg of hydrocortisone

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or 1.2 IU of ACTH just after the operation only once. The decapited foetuses as well as their non-decapited brothers (Controls from operated mother) were taken out under nembutal anaesthesia from the uterus at 0 h on the 31st day of pregnancy. Tissues were excised immediately from the foetuses and kept frozen for short time in chilled KCl 0.9%. There were slight differences in MAO activity of control foetuses from operated and non-operated mothers. COMT activity was exactly the same in both the controls studied. The new-born rats were adrenalectomized bilaterally under ether anaesthesia just after the birth. The tissues from normal and adrenalectomized young rats were excised 10 days after the operation. 15 male Sherman rats, weighing between 275 to 325 g and 8 weeks old, were administered with 75 mg of metopirone in olive oil i.p. The tissues were dissected out 8 h after the injection of metopirone. All the tissues were conserved in ice-cold KCl 0.9% before the assays of MAO and COMT.

Assays of MAO and COMT: MAO was assayed in total tissue homogenate while COMT was assayed in the supernatant of the tissues[19–21]. Tryptamine-^{14}C-bisuccinate, as a substrate for MAO, and S-Adenosyl Methionine-^{14}C, as a methyl donner for COMT, were utilized[19,21]. The activity of MAO is expressed in dpm per mg of tissue directly as toluene extration media represented proportionality between dpm-extracted and micro moles of ^{14}C-indole acetic acid transformed during 20 min of incubation. The activity of COMT in the supernatant of the tissues was measured by our modified method[20]. The entire assay was the same as in the original method of AXELROD[21], except that the tissue homogenates were centrifuged at 6,000 $\times g$ for 30 min. This modification did not change the precision of the assay to the slightest extent. The results of enzyme COMT are expressed in dpm/g of tissue. The enzyme activity was linear with dpm extracted in the range of our tissue concentrations added to the incubation mixture. In vitro the effects of hydrocortisone on partially purified hepatic MAO and COMT were also studied[22]. The solution of hydrocortisone in water from concentrations of 0.5 µmole to 2 µmole was added directly to the incubation mixture of MAO and COMT. The statistical differences are shown with Student-*t*-Test[23]. The results are expressed with standard error of the mean values.

Results: Table I shows the effects of foetal decapitation at the age of 20 days on the activities of enzymes MAO and COMT. The MAO activity expressed in dpm/mg of tissue was slightly higher in controls from operated mother than in controls from unoperated mother, except in the lung. Following decapitation there were respective rises of 74%, 30%, 43% and 34% in adrenals, kidney, paraganglia (Extra adrenal chromaffin tissue) and lung compared to the control values. These rises were highly significant (control adrenal and decapited adrenal, $P < 0.001$; control kidney and decapited kidney, 0.001

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Table I. Activity of enzyme MAO in tissues of decapited rabbit foetuses in dpm/mg of tissue

Treatment group	Adrenals	Kidney	Paraganglia	Lung
MAO in controls from unoperated mother	350 ± 20 (6)	560 ± 18 (12)	485 ± 18 (6)	715 ± 35 (6)
MAO in controls from operated mother	420 ± 23 (9)	598 ± 20 (19)	493 ± 40 (11)	705 ± 38 (13)
MAO in decapited at 20 days	610 ± 40 (11)	723 ± 33 (11)	693 ± 78 (8)	955 ± 23 (11)

()Number of determinations.

Table II. Activity of enzyme COMT in dpm/g of tissue

Treatment group	Lung	Heart	Kidney	Adrenals
COMT in controls from operated mother	49640 ± 3083 (5)	3780 ± 92 (5)	50000 ± 2040 (4)	5040 ± 140 (10)
COMT in decapited at 20 days	71160 ± 3100 (5)	4230 ± 95 (5)	58200 ± 3940 (5)	5600 ± 24 (10)

() Number of determinations.

Table III. Effects of adrenalectomy at birth on activity of enzyme MAO in dpm/mg of tissue in new-born rats

Treatment group	Heart	Hypophysis	Hypothalamus
MAO in 10-day-old rats	7500 ± 154 (12)	1665 ± 51 (13)	4138 ± 208 (12)
MAO in 10-day-old rats adrenalectomized at birth	10200 ± 260 (12)	4465 ± 140 (13)	4400 ± 60 (12)

() Number of determinations.

$P < 0.005$; control paraganglia and decapited paraganglia $P < 0.025$; control lung and decapited lung, $P < 0.001$).

Table II indicates activity of COMT in foetuses decapited at the age of 20 days in dpm/g of tissue. The enzyme activity was 43%, 11%, 16% and 11% higher from control values respectively in lung, heart, kidney and adrenals. The increases in lung and heart were statistically significant from their respective controls ($P < 0.01$, $P < 0.05$).

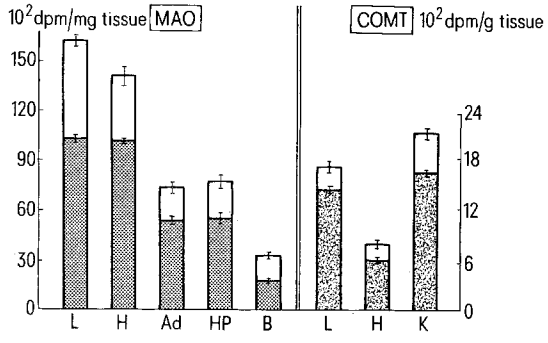
The effects of adrenalectomy at birth on activity of MAO are given in Table III. The heart and hypophysis showed high rises of 33% and 167% from controls 10 days following adrenalectomy ($P < 0.001$). Hypothalamic MAO increased slightly but without any statistical significance.

The Figure illustrates the effects of glucocorticoid inhibition on activity of MAO (dpm/mg fo tissue) and COMT (dpm/g of tissue) in different organs. The tissues were excised 8 h after the administration of metopirone. MAO activity in liver, heart, adrenals, hypohysis and brain was 58%, 38%, 38%, 41%, 98% higher than their controls respectively. These rises showed high statistical significance in all the organs ($P < 0.001$).

The administration of 1.5 mg of hydrocortisone or 1.2 IU of ACTH produced declines in activities of MAO and COMT in adrenals, kidneys and paraganglia of decapitated foetuses. The effects of ACTH administration were more profound than hydrocortisone. The range in declines in enzyme activities varied from 20% minimum to 48% maximum.

Discussion. The results of the present investigation provide evidence that glucocorticoids inhibit MAO and COMT activities in vivo as well as in vitro. The ablation of hypophysis in adults as well as in the foetus[24,25] affects the hormones of the adrenal cortex severely and results in its eventual atrophy. The inactivation of the foetal adrenal cortex by decapitation supports our hypothesis, as there were significant rises in MAO and COMT activities just 10 days after the operation. The MAO activity in young adrenalectomized rats confirms our results in decapited foetuses, as adrenalectomy caused highly marked rises in MAO activity in this short interval of time. The experiments in adult rats with administration of metopirone show clearly that decline in glucocorticoid concentration is followed by rises in MAO and COMT activities. Metopirone is a strong inhibitor of corticoidogenesis and it is well established that it reduces cortisol production right after its administration[16]. Its effects are nearly maximal for 6 to 8 h as cortisol concentration falls to zero[18]. The rises in MAO and COMT activities seem to be inversely proportional to the amount of glucocorticoids present in the body. The results in vitro support our findings in vivo as hydrocortisone in low concentrations inhibited both the enzymes of catecholamine degradation. Previous studies on the role of cortical hormones in adrenaline biosynthesis seem absolutely contrary in catecholamine degradation[3,10]. The observations of WURTMAN and AXELROD[8] also show that administration of potent synthetic glucocorticoid, dexamethasone also reduces the activities of MAO and COMT. The inactivation or ablation of adrenal steroidogenesis affects catecholamine metabolizing enzymes. The foetal decapitation in rabbits produced atrophy of adrenal cortex resulting in lower levels of corticoids. This deficiency could not be overcome by the passage of maternal corticoids to the foetus. Maternal adrenalectomy does not change the storage of adrenaline and noradrenaline in adrenals of decapited foetuses[26]. The replacement therapy with hydrocortisone or ACTH supplemented the deficiency of glucocorticoids and reduced the activities of enzymes MAO and COMT in rabbit decapited foetuses.

The molecular mechanisms by which the glucocorticoids inhibit the activities of enzymes MAO and COMT could be referred to extensive studies of LITWACK and SINGER[27], FEIGELSON and FEIGELSON[28] on the subcellular actions of these hormones. There is abundant evidence available that glucocorticoids regulate many enzymes of metabolic significance by induction of protein synthesis of new enzyme molecules[29–31]. The exact connection between receptor sites for glucocorticoids and their effects on protein synthesis remains tenuous and subject to further extensive studies at genetic level. Now it is generally accepted that these hormones probably exert their effects at the level of RNA transcription to DNA[32]. Previous studies also provide evidence that changes in enzyme activity are thought to occur on the amount of enzyme protein present in the tissues and consequently the regulatory factors. The regulatory factors affect the rate of synthesis as well as break down of enzyme protein[33]. The biosynthesis of active enzyme protein might eventually be dependent on a sufficient amount of hormonally stimulated effectors or other cofactors[34].



The activities of enzymes MAO and COMT 8 h after inhibition of glucocorticoids with 75 mg of metopirone. The enzyme activities are expressed in dpm/mg or dpm/g of tissue. The tinted columns show the control values while the transparent part represents the increase. The mean values are given with standard errors (I—I). All the groups consisted of 10 rats. L, Liver; H, Heart; Ad, Adrenals; HP, Hypophysis; B, Brain; K, Kidney.

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Our results suggest that presence of glucocorticoids act as a rate-limiting factor for catecholamine degradation. These findings may have important clinical implications in the treatment of neuroleptic disorders caused by neurotransmitter degradation and metabolism. The possible use of glucocorticoids in the treatment of the above diseases in future could replace several synthetic MAO inhibitors widely used in clinical practice inspite of their considerable side effects.

Résumé. L'inactivation du cortex surrénal par l'hypophysectomie provoque une augmentation des activités MAO et COMT. L'administration d'ACTH ou d'hydrocortisone réduit l'augmentation des activités enzymatiques chez les foetus décapités sacrifiés à 31 jours. Chez des jeunes rats de 10 jours, surrénalectomisés à la naissance, on constate une augmentation de l'activité MAO dans le cœur et l'hypophyse. Chez le rat adulte, l'inhibition de la synthèse des glucocorticoides par la métopirone est également suivie par une augmentation rapide et hautement significative de l'activité de ces deux enzymes dans la plupart des organes.

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The Effect of the Ergoline Derivative VUFB-6683 on the Adenohypophysial Prolactin Concentration in Rats

Some ergot alkaloids, and other compounds containing an ergoline or ergolene moiety, exert an antilactation effect which can be inhibited by prolactin[1–5]. The mechanism of action of these compounds is explained by their stimulation of the secretion of the hypothalamic prolactin-inhibiting factor (PIF)[6] and inhibition of prolactin release[7]. Another compound exhibiting lactation-inhibitory activity in lactating rats and dogs is the compound VUFB-6683, D-6-methyl-8-ergolin-I-ylacet-amide tartarate[8]. Concurrent administration of prolactin protected the lactation[9]. The preparation has a low toxicity; at a single oral administration to rats, the medium lethal dose LD_{50} equals about 1 g/kg of body weight. Our study has been aimed at finding how this preparation influences the adenohypophysial prolactin level.

Material and methods. Our experiments were performed on lactating Wistar rats whose postpartum weight was 200–220 g. Each mother was caged individually together with her litter, the size of which had been reduced immediately after birth to 6 puppies. Compound VUFB-6683 was administered by gavage in daily doses of 1.4 and 10 mg/kg for 4 consecutive days, and from the 4th day postpartum on in 5 ml/kg of a 2% aqueous solution of tartaric acid. Each experimental group had its control group receiving water by gavage. Throughout the duration of the experiment, the lactation was checked by body weight gain of the presence of milk-spots. On the 5th day the mothers were killed by decapitation 10 h after removal of the offspring.

Estimation of prolactin concentration. The hypophyses were removed, the anterior pituitary lobe isolated, and, after weighing, homogenized with redistilled water in a glass homogenizer. The prolactin concentration per 1 mg of wet adenohypophysis was estimated by the standard method of polyacrylamide disc electrophoresis[10,11]. The polyacrylamide concentration was 7.5% and electrophoresis was made with a current of 3 mA per tube. After conclusion of electrophoresis, the gels were stained overnight with 1% amido black 10B, and destained electrophoretically in 7% acetic acid in a device described by PRUSÍK[12]. The optical density (OD) of the prolactin zons was meas-

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Effect of VUFB-6683 on anterior pituitary (AP) prolactin concentration and weights in rats

Dosage of VUFB-6683	No. of rats	Prolactin concentration[a] (OD)	95% Confidence limite of means	Prolactin concentration[b] (IU)	AP weight (mg)
1 mg	14	0.42 ± 0.03	0.35–0.48	0.278[c]	6.0 ± 0.4[c]
Control	13	0.70 ± 0.03	0.64–0.76	0.459	8.4 ± 0.8
4 mg	7	0.33 ± 0.03[c]	0.27–0.39	0.279[c]	5.0 ± 0.5[c]
Control	7	0.56 ± 0.03	0.48–0.64	0.336	8.3 ± 0.2
10 mg	10	0.31 ± 0.09[c]	0.24–0.38	0.271[c]	6.7 ± 0.5[c]
Control	9	0.57 ± 0.02	0.53–0.61	0.369	8.2 ± 0.6

Mean values $\pm$ standard error are given. [c] Significant difference against control value ($p < 0.05$). [a] Prolactin concentration per 1 mg wet adenohypophysis expressed in terms of optical density and [b] in terms of international units of prolactin.