Table I. Dose-dependent effects of 4-pentynoic acid on hepatic TAT, hepatic glycogen, plasma glucose, and plasma corticosterone

Dose (mg/kg, s.c.)	Hepatic TAT (μmoles/min/g)	Hepatic glycogen (mg/g)	Plasma glucose (mg/100 ml)	Plasma corticosterone (μg/100 ml)
0	0.63 ± 0.10	8.00 ± 0.95	90 ± 4	19.9 ± 4.6
3.2	0.65 ± 0.10	16.82 ± 2.37[a]	117 ± 14	33.8 ± 1.0[a]
5.6	1.16 ± 0.13[a]	12.82 ± 1.12[a]	91 ± 2	47.6 ± 1.4[a]
10.0	2.19 ± 0.32[a]	3.64 ± 1.15[a]	54 ± 10[a]	41.3 ± 3.3[a]
17.8	1.90 ± 0.20[a]	1.93 ± 0.15[a]	30 ± 11[a]	51.0 ± 3.6[a]

[a] Significantly different from zero dose control, $P < 0.05$. All parameters were measured at 3 h except for plasma corticosterone levels, which were measured at 1 h. Mean values with standard errors for 5 rats per group are shown.

Several connections between the biochemical changes shown in Figure 2 and the elevation of hepatic TAT (Figure 1) are possible. First, and of primary interest to us, was the possible relation between the hypoglycemia and the elevation of enzyme activity. Second, the fall in hepatic glycogen content might also be related to the enzyme change. PERAINO et al.[11] suggested a reciprocal relationship between hepatic glycogen stores and amino acid catabolizing enzymes. They offered the teleological explanation that depletion of hepatic glycogen would create a demand for amino acid catabolism for the purpose of fulfilling existing energy requirements, and they entertained the possibility that glycogen might itself repress the synthesis of such enzymes. Third, glucocorticoids induce hepatic TAT, and the elevation of TAT after 4-pentynoic acid might have resulted from the elevation of corticosterone. Considering these possibilities, we determined whether a dose-response comparison could separate the effects of 4-pentynoic acid.

The Table shows the results with 4 different doses of 4-pentynoic acid. Surprisingly, glycogen stores were *increased* at the 2 lower doses and *decreased* at the 2 higher doses. Plasma glucose tended to increase at the low dose, and decreased at the 2 higher doses. Plasma corticosterone was elevated at all doses. Hepatic TAT was unchanged at the low dose and elevated at the 3 higher doses. These results argue against any direct relation between hepatic glycogen and TAT activity.

Hypoglycemia might lead to increased hepatic TAT levels via increased neural or hormonal input to the liver. Several lines of evidence suggest that neural control of hepatic TAT can occur[6,12–15]. Alternatively, hypoglycemia should result in increased release of hormones like glucagon, epinephrine, or glucocorticoids, and these hormones can elevate TAT. In an attempt to distinguish between the stimuli causing elevation of hepatic TAT after injection of 4-pentynoic acid, we measured its effect in adrenalectomized rats (Figure 3). Adrenalectomy completely prevented the elevation of TAT, showing that the enzyme effect was dependent on the adrenal glands and had been brought about by the secretion of adrenal hormones or by a steroid-dependent mechanism.

Zusammenfassung. 4-Pentinsäure, ein hypoglykämisches Mittel, vermehrte Leber-Tyrosinaminotransferase in intakten, nicht aber in adrenalektomierten Ratten.

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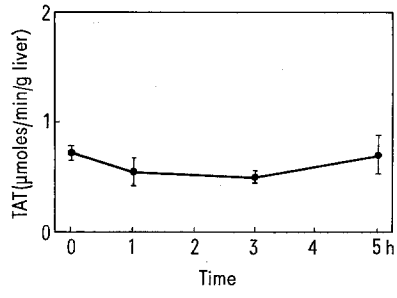

Fig. 3. Failure of 4-pentynoic acid to elevate hepatic TAT in adrenalectomized rats. All conditions as in Figure 1 except that the rats had been bilaterally adrenalectomized 8 days previously.

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Hormonal Influences on Erythrocyte Catechol-*O*-Methyl Transferase Activity in Humans

Catechol-*O*-methyl transferase (COMT) catalyses the transfer of a methyl group from S-adenosylmethionine to the hydroxyl of catecholamines, such as adrenaline or noradrenaline[1]. It is a soluble enzyme of erythrocytes[2], absent from plasma and platelets, but present in trace amounts in leukocytes[3]. Its major physiological function is inactivation of catecholamines in the circulation and in tissues with sparse adrenergic innervation[4].

Women with depression (primary affective disorder) have reduced erythrocyte COMT activity which cannot

be restored by psychiatric treatment, even if this is successful[5]. Women with other psychiatric conditions, together with men with depression, have normal red cell COMT[5]. Activity of COMT in rat liver is significantly reduced by estradiol, but not by progesterone or testerone[6].

The incidence of depression in women taking oral contraceptive preparations is about 6%, compared to only about 1 or 2% in women using other contraceptive methods[7,8]. Depression is one of the most frequent side-effects of oral contraceptives[9] and in one large survey[10] 28% of women who gave up oral contraceptives did so because of depression[11]. The biochemical etiology of depression is poorly understood, but a disturbance in catecholamine metabolism seems likely[12]. We have therefore investigated the effects of various exogenous steroid hormones on erythrocyte COMT activity in human subjets.

Blood was collected by venepuncture into heparinized tubes from subjects attending hospital or private clinics. A detailed history was taken from each. Plasma was removed after centrifugation at $2000 \times g$ and the packed cells carefully washed with 0.1 M phosphate buffer, pH 7.8, to remove any remaining plasma. The cells were again packed by centrifuge, buffer removed, and the cells lysed with ice-cold distilled water. The 'ghost' fraction was removed. Activity of COMT in the supernatant was measured using L-noradrenaline-D-bitartrate as substrate and S-adenosylmethionine-^{14}C-methyl as methyl donor[5]. After incubation at 37°C for 1 h, radioactive normetanephrine produced by the enzyme was extracted by isoamyl alcohol and measured in a liquid scintillation counter (Packard Tri-Carb). COMT activity was calculated as nanomoles (nM) ^{14}C-normetanephrine formed per hour by 1.0 ml packed erythrocytes.

We have studied various groups of subjects and mean values are given in Table I.

We found no significant difference between the mean values for erythrocyte COMT activity for normal men and women, nor could we find any effect of age or stage of the menstrual cycle. By taking blood at intervals from several individuals we found that the enzyme activity varied with time by only about $\pm$ 3%.

For psychiatric patients, COMT activity was normal in both men and women with schizophrenia, and in men with depression. However, women with depression had mean values significantly lower than normal ($P < 0.001$). There was no correlation between duration or severity of the depression and enzyme activity.

Erythrocyte COMT activity was significantly reduced also in women taking oral contraceptives ($P < 0.001$), but not in women treated by a depot progestogen as a long-acting contraceptive. Significantly low mean values were found in a group of 12 women who had received a 21-day course of oral estrogen (50 µg daily mestranol) and in a second group of 5 women who had taken a 21-day course of oral progestogen (1.0 mg daily norethisterone acetate). Normal enzyme activity was found in women receiving oral corticosteroids (prednisone at various doses) and in men taking an oral androgen (100 mg daily mesterolone).

In Table II we have presented the results on the women taking oral contraceptives by giving the numbers of subjects who had erythrocyte COMT activity less than 1 and 2 standard deviations (S.D.) below the mean value for the control group of normal women. We have broken down the contraceptive group depending on the progestogen component of the particular product used. It will be seen that over half the women taking oral contraceptives had COMT activities less than the control mean minus one S.D., while 16% of these women had enzyme values less than the mean minus two S.D. The small group of women taking oral progestogen alone show similar results, but women treated by the depot progestogen (MPA = medroxyprogesterone acetate, 150 mg in oil by i.m. injection every 3 months) had a more normal distribution of results.

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Table I. Erythrocyte COMT activity in humans

Group of subjects	Number in group	Mean COMT activity (nM/h/ml)	S.D.
Normal men	15	1.51	0.35
Normal women	36	1.48	0.33
Women with depression	12	0.95	0.29
Women with schizophrenia	12	1.50	0.36
Men with depression	10	1.54	0.31
Men with schizophrenia	11	1.53	0.33
Women taking oral contraceptives	62	1.13	0.40
Women with depot progestogen	15	1.38	0.47
Women taking oral estrogen	12	1.11	0.49
Women taking oral progestogen	5	1.02	0.51
Women taking oral corticosteroid	6	1.43	0.52
Men taking oral androgen	5	1.49	0.50

Table II. Influence of progestogens on erythrocyte COMT activity

Group of subjects	Progestogen	Number in group	Number of results below mean value of control group	
			Below mean minus 1 SD	Below mean minus 2 SD
Normal women	none	36	5 (14%)	1 (3%)
Women taking oral contraceptives	all	62	32 (52%)	10 (16%)
	NG	24	12 (50%)	5 (21%)
	EDA	10	5 (50%)	2 (20%)
	LE	10	5 (50%)	1 (10%)
	NEA	15	9 (60%)	2 (13%)
Women with depot progestogen	MPA	15	3 (20%)	2 (13)%
Women taking oral progestogen	NEA	5	2 (40%)	1 (20%)

NG, norgestrel; EDA, ethynodiol diacetate; LE, lynestrenol; NEA, norethisterone acetate.

To determine whether oral contraceptives induce low erythrocyte COMT activity we studied 9 women before they started taking oral contraceptives, and again after 1 to 3 months of treatment. Results are shown in Table III. Six of the 9 showed reductions from 10 to 44% post-treatment, while the mean decrease in enzyme activity for the group was 15%.

Finally, we studied groups of women in different stages of pregnancy with the results shown in Table IV. Enzyme activity during the 1st trimester is not significantly different to that of non-pregnant women, but a significant reduction occurs during the 2nd and 3rd trimester ($P < 0.05$ and < 0.001, respectively). Erythrocyte COMT activity remains low during the first 7 days post-partum.

As we have been unable to inhibit COMT activity in vitro with natural or synthetic steroid hormones added at $10^{-4}M$ concentration, we feel that hormones act to reduce formation of COMT during erythropoesis. After the first 3 months, there is no correlation between COMT activity and duration of use of oral contraceptives, but there is a progressive decrease in this initial period.

Reduced erythrocyte COMT activity would be excepted to increase the concentration of circulating catecholamines and serum noradrenaline is known to be high in women with depression[5]. If COMT of brain is similarly affected, alterations in catecholamine metabolism will occur and could be of importance in depression.

It seems likely that the high incidence of depression in women taking oral contraceptives, or during the post-partum period, is related to hormonal suppression of erythrocyte COMT activity. As only about half of the women we studied showed this enzyme reduction, determination of the enzyme might be of value in screening women liable to develop depression in response to oral contraceptives or pregnancy.

Table III. Influence of oral contraceptives on erythrocyte COMT

Group of subjects	Erythrocyte COMT activity (nM/h/ml) Pre-treatment	Post-treatment	Change (%)
Women taking oral contraceptives	1.76	0.98	−44
	1.44	1.05	−27
	1.18	1.16	− 2
	1.58	1.62	+ 3
	1.55	1.39	−10
	1.17	0.97	−17
	1.71	1.50	−12
	1.50	1.52	+ 1
	1.66	1.15	−31

Table IV. Erythrocyte COMT activity during pregnancy

Group of subjects	Number in group	Erythrocyte COMT activity (mean nM/h/ml)	S.D.
Normal women	36	1.48	0.33
Pregnant women	35	1.19	0.35
First trimester	7	1.40	0.39
Second trimester	15	1.20	0.40
Third trimester	13	1.08	0.32
Post-partum 7 days)	10	1.15	0.38

Résumé. Dans le cas de beaucoup de femmes prenant des contraceptifs oraux, l'activité du transférase catéchole-*O*-méthyle du globule rouge est réduite à des nivaux analogues à ceux observes dans le cas de femmes souffrant de dépression non-traitée. Le dépôt de contraceptifs à action durable n'affecte pas d'une manière significative l'enzyme, bien qu'elle soit réduite pendant le troisième trimestre de la grossesse.

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A Comparative Study of Nuclear Proteins in Chick Embryo Cells and Their Primary and Secondary Fibroblasts in Culture

Chick embryo cell cultures have been used extensively in many studies. However, the limited quantity of cultured cells constitutes an experimental disadvantage in studies dealing with the isolation of nuclear proteins. The ideal is to use a minimum amount of available radioactively labeled cultured cells which are subsequently mixed with readily available embryo cells serving as a carrier for the isolation of radioactively labeled cultured cell components. Nevertheless, the possible changes of some cellular parameters during the passage of embryo to cultured cells cast some doubt on the methodological validity of using uncultured embryo cells as carrier for components from cultured cells. For these reasons we have carried out a comparison of nuclear proteins of chick embryo cells and of cultured primary and secondary cells derived from chick embryo using polyacrylamide gel electrophoresis.

Materials and methods. Embryos were removed from 10- to 11-day-old fertilized eggs obtained from Spafas Inc. The viscera, limbs and head were discarded. The remaining parts were homogenized at 4 °C in 5 volumes of 0.05 M *Tris* buffer, pH 7.4, containing 0.32 M sucrose and 3 mM $MgCl_2$. Purified nuclei were prepared as described previously[1].

Primary and secondary cell cultures were prepared from embryos according to the method described by VOGT[2]. Approximately 4×10^7 cells were added to Roux bottles and incubated at 37 °C. The cells usually growing to confluency within 3 to 5 days were designated as primary cell culture. Secondary cells were produced by the passage of primary cells[2]. Isolation of nuclei from primary and secondary cells (5×10^9 cells per experiment) was accomplished as described above for embryo nuclei.

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