Distribution of the dose rate in mrad/h in several organs of rats

Organ	Dose rate in mrad/h	
	Series 1 Rn: 12.5 nCi/l RaB/Rn: 0.25	Series 2 Rn: 110 nCi/l RaB/Rn: 0.33
Blood	1.07	11.9
Liver	0.64	6.9
Kidney	3.50	40.2
Adrenal glands	0.62	6.0
Red marrow	0.35	3.6
Muscle	0.75	8.2

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Results. The Figure represents the corticosteroid concentrations as time-functions for both series of measurements. In both curves a first peak appears after 8 h, a second maximum with the higher ^{222}Rn concentration after 5 days, with the lower concentration after 9 days. Both maxima are statistically significant. One can see that the hormone production is stimulated as an impulse, followed by a typical 'tail', as is observed in certain stress situations[5]. Especially interesting is the occurence of a second increase. The following decrease, however, is not statistically significant and would require prolonged inhalation studies. Both curves show the occurrence of a perturbation in the corticosteroid level as a response to the radiation effects. The differences between the high and the low dose could probably be interpreted as the appearance of a phase difference in an re-equilibration process. The 2 maxima of the corticosteroid level indicate, moreover, that the intracellular controls are switched in 2 steps. Further studies on this problem are in progress.

Hormonal Induction of Glutamate Dehydrogenase in Rat Liver[1]

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Summary. Glutamate dehydrogenase rapidly increases in microsomes and appears in the cytoplasm after administration of cortisone, cAMP, hydrocortisone-acetate. Prolonged administration of ACTH maintains high level of enzyme in the mitochondria and microsomes. Hydrocortisone-acetate, insulin and corticosterone decrease drastically enzyme in mitochondria.

Glutamate dehydrogenase (GDH) (EC 1.4.1.3), though a mitochondrial enzyme, is synthesized in the ribosomes. SOLOMON[2] reported that enzyme activity in embryonic chicken liver could be found only in the cytoplasm. It began to increase in the mitochondrial fraction after the 12th day of incubation and diminished drastically in the supernatant after 16 days of incubation. Recently, GODINOT and LARDY[3] measured enzyme activity in isolated rat liver microsomes and identified the enzyme by using antibodies against GDH and polyacrylamide gel electrophoresis. It was suggested also that glutamate dehydrogenase is transported into mitochondria in combination with cardiolipin[4]. Previous studies on GDH induction by hormones are summarized in Table I. These

Table I. Survey of the literature on induction of glutamate dehydrogenase in rat liver homogenate

Hormone or treatment	Effect on GDH level	Reference
Starvation	+	6,7
	0	3
Protein-free diet	+	8,9
High protein diet	+	7
High glucose diet	—	10
Alloxan diabetes	+	11
	0	12
Alloxan diabetes + insulin	+	11
Alloxan diabetes + glucagon	+	11
Adrenalectomy	—	10, 13–15
Adrenalectomy + cortisol	0	11
Adrenalectomy + cortisol + D-aldosterone	0	15
Hypophysectomy	—	16–19
Glucagon	+	11
Cortisol	+	10
Corticosterone	+	10
Deoxycorticosterone	0	10

Enzyme activity measured in crude homogenates (supernatant after $900 \times g$) suspended in water. +, increase in enzyme level; —, decrease in enzyme level; 0, no effect on enzyme level.

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Table II. Glutamate dehydrogenase activity in subcellular fractions of rat liver in short term experiments

Experiment	Dose	Mitochrondria (μmole NADH/min/g protein)	Microsomes	Cytosol
3 h after hormone administration				
Control		76.0 ± 10.7 (7)	0.6 ± 0.3 (4)	0.0 ± 0.0 (5)
Corticosterone	5 mg/kg	95.1 ± 30.0 (4)	0.9 ± 0.5 (4)	0.3 ± 0.1 (4)
Corticosterone	25 mg/kg	71.6 ± 11.3 (5)	0.3 ± 0.2 (5)	0.4 ± 0.2 (5)
Cortisone	25 mg/kg	70.7 ± 40.0 (4)	1.6 ± 0.4 (4)	1.3 ± 0.3 (4)
Cortisone	50 mg/kg	76.3 ± 9.1 (5)	1.4 ± 0.4 (5)	1.3 ± 0.3 (5)
ACTH	100 IU/kg	82.8 ± 12.8 (3)	0.6 ± 0.1 (3)	0.3 ± 0.1 (3)
c-AMP	2.5 mg/kg	63.6 ± 5.6 (3)	6.1 ± 2.3 (3)	1.2 ± 0.3 (3)
5 h after hormone administration				
Control		76.0 ± 10.7 (7)	0.6 ± 0.3 (4)	0.0 ± 0.0 (5)
Cortisone	50 mg/kg	84.2 ± 3.6 (3)	1.7 ± 0.4 (3)	1.2 ± 0.3 (3)
ACTH	100 IU/kg	96.1 ± 24.1 (4)	0.8 ± 0.2 (4)	1.1 ± 1.0 (4)
Hydrocortisone-acetate (cortisol)	100 mg/kg	21.1 ± 7.6 (3)	1.7 ± 1.3 (3)	0.2 ± 0.1 (3)

studies were performed with crude homogenates suspended in water. The present study presents results of in vivo studies on GDH induction in various subcellular liver fractions after the administration of hormones to albino rats.

Materials and methods. ACTH was purchased from Nutritional Biochemical Corporation and corticosterone, c-AMP, hydrocortisone (cortisol), insulin, cortisone, sucrose, *Tris*, EGTA, lubrol from Sigma Chemical Co. Other chemical were obtained from J. T. Baker Chemical Co.

Induction of enzyme in vivo. Hormones (dissolved in appropriate solvent, ethanol or 0.9% NaCl) were administered i.p. to albino male rats (200–250 g body wt.) fed ad libitum. In short term experiments the rats were sacrificed a few hours after hormone administration; in long term experiments hormones were administered every day and the rats were sacrificed the day immediately following the last injection. Control rats were treated same as the experimental rats but received injections of hormone vehicle only.

Liver fractionation. The rat livers were homogenized (10% homogenate) in 0.25 *M* sucrose, 10 m*M* *Tris*-HCl, pH 7.4, 1 m*M* EGTA-*Tris*, pH 7.4, and centrifuged at 800 × *g* for 10 min. Mitochondria were isolated by centrifugation of the supernatant at 8,000 × *g* for 10 min and microsomes were isolated last by centrifugation at 105,000 × *g* for 30 min. The supernatant from the last centrifugation was used as the cytosol preparation. Isolated liver fractions – mitochondria, microsomes – were suspended in 10 m*M* *Tris*-acetate buffer, pH 7.4, and sonified for 2 min in a Heat System Sonifier at 80 watts.

Measurement of glutamate dehydrogenase activity. GDH activity in mitochondria, microsomes and cytosol was measured by following NADH oxidation at 340 nm in the presence of NH_4Cl and α-ketoglutarate. The incubation medium (3 ml final volume) in the cuvette contained 50 m*M* potassium phosphate buffer, pH 7.6, 0.1 ml of 0.1% lubrol (tc dissolve particles), 53 m*M* ammonium chloride, and 0.1 m*M* NADH. Absorbancy at 340 nm was followed for about 2 min and then the reaction was started by the addition of 3.3 m*M* α-ketoglutarate-*tris*. Linear initial velocities were calculated as μmole NADH/g protein/min and corrected for a nonspecific decrease in NADH absorbency (very slow) before the addition of α-ketoglutarate.

Protein determination. Protein in liver fractions was measured with the biuret method as described by LAYNE[5].

Results. Results of the experiments on the induction of glutamate dehydrogenase in liver cellular fractions by corticosterone, ACTH, c-AMP, hydrocortisone, cortisone and insulin are presented in Tables II and III.

Table III. Glutamate dehydrogenase activity in subcellular fractions of rat liver in long term experiments

Experiment	Dose	Mitochrondria (μmole NADH/min/g protein)	Microsomes	Cytosol
Administration of hormone for 3 days and sacrifice on the 4th day				
Control		76.1 ± 10.2 (7)	0.6 ± 0.3 (4)	0.0 ± 0.0 (5)
Hydrocortisone-acetate (cortisol)	25 mg/kg	13.1 ± 2.0 (5)	0.6 ± 0.3 (5)	0.8 ± 0.1 (5)
Insulin	25 IU/kg	9.9 ± 1.0 (6)	0.2 ± 0.3 (6)	0.2 ± 0.3 (6)
Administration of hormone for 5 days and sacrifice on the 6th day				
Control		76.1 ± 10.7 (7)	0.6 ± 0.3 (4)	0.0 ± 0.0 (5)
Corticosterone	25 mg/kg	28.1 ± 0.2 (3)	1.4 ± 0.1 (3)	0.4 ± 0.1 (3)
Cortisone	25 mg/kg	71.1 ± 7.4 (3)	1.2 ± 0.3 (3)	1.2 ± 0.3 (3)
ACTH	100 IU/kg	93.1 ± 10.3 (3)	1.5 ± 0.2 (3)	0.0 ± 0.0 (3)
Hydrocortisone-acetate (cortisol)	25 mg/kg	27.9 ± 0.7 (3)	1.1 ± 0.1 (3)	3.8 ± 1.1 (3)

Enzyme activity increases by 100% in microsomes 3 h after administration of cortisone and appears in the cytosol. 5 h incubation leads to a 10% increase of enzyme in mitochondria also. Prolonged administration of cortisone for 5 days maintains a 100% increased level of enzyme in the microsomes and in cytosol; its level in mitochondria returns to normal. Corticosterone induces enzyme activity in the cytosol; after a 5 day prolonged administration, there is a 60% decrease of enzyme activity in mitochondria and a 100% increase in microsomes. Hydrocortisone-acetate (cortisol) induces a rapid 70% decrease of enzyme level in mitochondria after 5 h, causes its appearance in the cytosol and, after a 5 day prolonged administration, the enzyme level in microsomes increases by about 100%. In the cytosol the enzyme is maintained at a very high level. ACTH at first increases the enzyme level in mitochondria (by about 10%) and causes its appearance in the cytosol. Prolonged administration of the hormone maintains a high level of enzyme in the microsomes (about 100%) and in mitochondria (about 20%). c-AMP causes a rapid 10-fold increase of enzyme activity in the microsomes, appearance of the enzyme in the cytosol with a 20% concomitant decrease in mitochondria. Insulin administered for 3 days decreases the enzyme level in mitochondria by about 90% and in the microsomes by about 70%; it produces a slight appearance of the enzyme in the cytosol (if any).

Discussion. In former studies, summarized in Table I, GDH activity was measured in crude liver homogenates in water after centrifugation at 900 $\times g$. It can be seen that GDH level is controlled by glucocorticoids, the hypophysis, D-aldosterone, and the adrenal glands. Relative to the effect of diet, starvation and diabetes, controversial results were reported. All reported changes in enzyme activity were within the range of 15–20%. In our experiments, the enzyme level is relatively stable in mitochondria. It increases only after a prolonged administration of ACTH, and decreases after the administration of insulin, cortisol and corticosterone. These effects seem to be secondary and associated with the transport of the enzyme from microsomes and with changes in permeability of the mitochondrial membrane. The most dramatic and rapid increases in enzyme level are observed for microsomes after administration of cortisone, and c-AMP with a concomitant appearance of the enzyme in the cytosol. In long-term experiments, corticosterone, cortisone, ACTH and cortisol maintain a high level of enzyme in microsomes and cytosol. Especially interesting is the high level of enzyme in the cytosol following the prolonged administration of cortisol. It was reported that the half-life of specific proteins in ribosomes, nuclei, cytosol and mitochondria (including GDH as a representative enzyme of the soluble mitochondrial fraction) is about 5 days [20, 21]. Thus it seems unlikely that the observed changes in enzyme activity in long term experiments are due also to the differences in the turnover of the enzyme in various subcellular fractions. The results presented suggest that the control of enzyme biosynthesis is located between the nucleus and the microsomes. Cortisol, corticosterone, insulin and ACTH (or other hormones increased by ACTH) may have additional effects on the transport of the enzyme and on the permeability of the mitochondrial membrane.

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On the Time Course of Thyrotropin Suppression by High Doses of Thyroid Hormones[1]

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Summary. Basal and stimulated TSH decreased progressively. Basal TSH was suppressed below the detection limit of 0.4 μU/ml after 74 h in 2 of the T_3 and all of the T_4 treated individuals. At this time, in both groups 3 individuals could be significantly stimulated by TRH (about 5% of the pretreatment stimulation). There was no significant difference in the time course of suppression obtained by T_3 or T_4, though plasma T_3 levels in the T_4 treated group were considerably lower.

Suppression of basal and TRH-stimulated TSH secretion in thyrotoxicosis or by artificial elevation of plasma thyroid hormone levels is well-documented. The relative contribution of triodothyronine (T_3) and thyroxin (T_4) to this process is not yet established. There are also discrepancies which concern the time course of suppression of the thyrotrope. SNYDER and UTIGER[3] found an almost complete abolishment of the TRH-stimulated TSH secretion by a 4-week treatment with 30 μg T_3 and 120 μg T_4 daily. SHENKMAN et al.[4] saw a complete lack of responsiveness of the thyrotrope to TRH stimulation 1 h after ingestion of 50 μg T_3. AZIZI et al.[5], however, reported recently that the TSH response after TRH injection under these conditions is only minimally depressed compared to basal conditions.

Therefore we have investigated the time course of the basal and TRH-stimulated TSH secretion in normal subjects after ingestion of 50 μg T_3 every 12 h and after administration of a single dose of 3.0 mg T_4 respectively. 100 μg T_3/d is used during the conventional suppression test whereas 3.0 mg T_4 as a single dose has been suggested more recently[6] for the same purpose.

We wanted to study the following questions: 1. How long does it take under these conditions to obtain a complete abolishment of the TSH response after TRH? 2. Is there any difference in the time course of pituitary

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