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COMPARATIVE EFFECTS OF DEXAMETHASONE AND PHENOBARBITAL ON ADRENAL CORTEX, LIVER CYTOCHROME P450 CONTENTS AND SERUM THYROID HORMONES

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SUMMARY

The effects of dexamethasone and phenobarbital on adrenocortical mitochondria and microsomes, and on liver microsomes were investigated in the rat. Dexamethasone injection (300 μg/100g s.c. twice a day for 2 days) increased by 32% liver wt. and decreased by 14% adrenal wt. Cytochrome P450 contents, both in liver microsomes and in adrenal cortex mitochondria and microsomes, were decreased by about 20 to 25%. Phenobarbital treatment (5mg/100g i.p. twice a day for 2 days) increased both liver and adrenal wt. by 26 and 35% respectively. It induced an increase in liver microsomal cytochrome P450 content, but no change was observed in adrenal cortex mitochondria and microsomes. The effects of dexamethasone and phenobarbital on deiodinating systems located in liver microsomes were indirectly studied by the estimation of serum concentrations of 3,5,3′-triiodo-L-thyronine (T_3), L-thyroxine (T_4) and 3,3′,5′-triiodo-L-thyronine (rT_3). Dexamethasone administration slightly diminished T_4 concentration and increased rT_3 concentration without a correlative decrease of T_3 in serum. Phenobarbital decreased T_4 and T_3 serum concentration however rT_3 was unchanged.

INTRODUCTION

Adrenal cortex and liver catalyse hydroxylations of many different kinds of substrates including cholesterol and steroids [1–3]. Hydroxylating enzymes are located either in microsomes of various tissues or in mitochondria especially in adrenal cortex, and require molecular oxygen and a cytochrome P450 as a terminal oxidase [4, 5]. These requirements are characteristic of the group of enzymes classified as mixed-function oxidases [6]. In liver microsomes, these enzymes promote detoxication of various drugs such as phenobarbital. The hydroxylation of the drugs was followed by induction of several enzymes in hepatic microsomes such as NADPH cytochrome c reductase and cytochrome P450 [7, 8]. Dexamethasone decreases corticotrophin secretion which causes a drop in corticosterone secretion by the adrenal gland [9] and a significant reduction in circulating concentration of 3,5,3′-triiodo-L-thyronine [10]. Nothing is known of the effects of administration of phenobarbital on adrenal cortex hydroxylation and of dexamethasone reactions in liver.

The present study is designed to compare the effects of both treatments on adrenal cortex mitochondria and microsomes and on liver microsomes.

MATERIALS AND METHODS

Experiments were performed on normal male Wistar rats weighing about 200 g. Some of the animals were given either dexamethasone 300 μg per 100 g BW s.c., twice a day for 2 days, or phenobarbital 5 mg per 100 g BW i.p., twice a day for 2 days. The animals were killed 24 h after the last injection.

Isolation of particles

Mitochondria. Each assay required a total of 40 normal or treated rats. They were killed by carotid section. The adrenals were removed, weighed and carefully dissected to eliminate the medulla. The amount of adrenal cortex tissues obtained was 0.5–1 g. Mitochondria were isolated in 0.33M sucrose using the method of Nakamura and Tamaoki[11]. The homogenate was centrifuged twice for 15 min, once at 600 *g* and once at 5000 *g*. The pelleted mitochondria were washed twice with 0.33M sucrose and centrifuged for 15 min at 5000 *g*. Mitochondria were checked for quality by polarography which was used to determine oxygen uptake and phosphorylation.

Protein analysis was carried out using the tech-

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Table 1. Liver and adrenal glands weights in normal, dexamethasone and phenobarbital treated rats

Treatment	Liver (g/100 g BW)	Adrenal glands (mg/100 g BW)
Control	5.42 ± 0.26 (14)	17.53 ± 1.25 (10)
Dexamethasone	7.0 ± 0.83* (14)	15.07 ± 1.10 (10)
Phenobarbital	6.85 ± 0.29** (14)	23.65 ± 2.15* (10)

Values are the mean ± S.E.M. The number of experiments is given in parenthesis. *$P < 0.01$. **$P < 0.001$.

nique described by Lowry *et al.*[12] and Gornall *et al.*[13]; 6 to 8 mg of mitochondrial proteins were obtained from each preparation.

Microsomes. The mitochondrial supernatant of adrenal cortex preparation was centrifuged at 27,000 *g* for 15 min to eliminate light mitochondria and chromafine particles. Microsomes were sedimented for 1 h at 105,000 *g* and resuspended in 0.25M sucrose. Protein was measured as before [12, 13]; 8–10 mg of microsomal proteins were obtained from each preparation. Liver microsomes were isolated after homogenization in 0.25M sucrose. After elimination of mitochondria at 8000 *g* [14] and at 27,500 *g*, the supernatant was centrifuged at 105,000 *g* for 1 h. Sedimented microsomes are resuspended in 0.25M sucrose and protein estimated [12, 13].

Determination of cytochrome P450 by spectrophotometry

Cytochrome P450 was determined by differential spectra according to the method of Omura and Sato[15]. Mitochondria or microsomes (about 2 mg) were suspended in 1 ml 0.1M phosphate buffer (pH 7.0). A few crystals of sodium dithionite ($Na_2S_2O_4$) were added to reduce possible traces of blood pigment. The content of one cuvette was then gassed with CO which forms a P450–CO complex (CO-Fe^{++}). The concentration of cytochrome can be calculated by using the extinction coefficient difference between 450 nm (peak) and 490 nm which is 91 cm^{-1} mM^{-1}.

Polyacrylamide gel electrophoresis. Electrophoresis was performed in polyacrylamide gel (8.5% total acrylamide concentration, 0.22% methylene bis-acrylamide to acrylamide, 0.075% ammonium peroxodisulfate and 0.15% tetramethylenediamine) in 0.01M sodium phosphate (pH 7.0) with 0.1% sodium dodecyl sulphate and 0.1% mercaptoethanol [16]. Samples containing 50–100 μg protein, in the buffer used for electrophoresis were submitted to 8 mA/tube (90 × 6 mm) for 6 h. Gels were stained overnight with 0.04% Coomassie blue (w/v), 45% methanol (V/V), 9% acetic acid (V/V) and destained with 5% methanol and 7% acetic acid. Photometric measurements of the stained gels were obtained in a Gilford gel spectrophotometer.

Molecular mass were determined by using pure marker proteins of commercial origin as follows (17): β-galactosidase (130,000 daltons) phosphorylase a (94,000 daltons), bovine serum albumin (68,000 daltons) creatine kinase (40,000 daltons), trypsin (23,300 daltons) and cytochrome *c* (11,700 daltons).

Radioimmunological assays of thyroid hormones

Thyroid hormones concentrations in serum were measured by radio-immunology in the case of 3,5,3′-triiodo-L-thyronine (T_3, Seralute Ames Kit) and 3,3′,5′-triiodo-L-thyronine (rT_3, Biodata hypolab Kit) and for 3,5,3′,5′-tetraiodo-L-thyronine (T_4) by competition (Tetralute Ames Kit). The serum came from normal, dexamethasone and phenobarbital treated animals. Measurements were made on 7–8 batches of serum, each batch coming from three to four rats.

RESULTS AND DISCUSSION

Table 1 shows that dexamethasone treatment decreased the adrenal cortex wt. and increased the liver wt. This increase may be explained by a stimulation of protein synthesis in liver in the face of peripheral tissue breakdown as observed after cortisol administration [18]. The decrease of adrenal wt. may result from the inhibition of ACTH secretion by the synthetic steroid [9]. After phenobarbital treatment, both

Table 2. Content of cytochrome P450 in liver microsomes, adrenal cortex mitochondria and microsomes of normal, dexamethasone and phenobarbital treated rats

Treatment	Liver microsomes	Adrenal cortex	
		Mitochondria	Microsomes
Control	0.55 ± 0.09	1.78 ± 0.16	0.93 ± 0.14
Dexamethasone	0.41 ± 0.12*	1.47 ± 0.24**	0.73 ± 0.17*
Phenobarbital	1.49 ± 0.31**	1.90 ± 0.41	0.89 ± 0.15

Concentrations are expressed in nmol mg^{-1} mitochondrial or microsomal protein. Values are the mean of 6 experiments ±S.E.M. For each experiment 30–50 rats were used. *$P < 0.05$. **$P < 0.01$.

Table 3. Concentrations of serum iodothyronines in normal, dexamethasone and phenobarbital treated rats

Treatment	T_4 (ng/ml)	T_3 (ng/ml)	rT_3 (ng/ml)
Control	53.5 ± 4.7 (14)	1.13 ± 0.08 (15)	0.14 ± 0.06 (7)
Dex.	45.5 ± 5.2* (13)	1.02 ± 0.13 (14)	0.24 ± 0.09* (7)
PB	23.0 ± 7.2** (13)	0.69 ± 0.12** (14)	0.13 ± 0.05 (7)

Dex., dexamethasone; PB, phenobarbital. Values are the mean ± S.E.M. The number of experiments is given in parenthesis. *$P < 0.05$. **$P < 0.01$.

liver and adrenal cortex wt. increased (Table 1). The liver wt. increase is a classical phenomenon of phenobarbital treatment which induces enzymatic systems participating in many detoxicating processes in relation to hydroxylating mechanisms [19]. No such enzymatic induction has however been found in tissues such as muscle and brain [20]. The increase in adrenal cortex wt. is difficult to explain. It seems to be independent of an excess in ACTH secretion due to stress since cytochrome P450 contents were not changed (Table 2).

Liver microsomes contain not only hydroxylating enzymes but also iodothyronine deiodinases, since it has been found that thyroxine gives 3,5,3'-triiodo-L-thyronine in the presence of such liver preparations [21].

Table 3 shows that, after dexamethasone treatment, the serum concentration of T_4 decreased slightly without a concomitant decrease of T_3. rT_3 Concentration increased significantly. So, it is likely that the T_4 deiodinating microsomal systems are specifically activated in keeping with clinical results previously observed in man [22]. In the case of phenobarbital treatment, the simultaneous decrease of T_3 and T_4 without change in rT_3 concentration is due as in man [23, 24] to an hepatic accumulation of T_4 as previously observed in the rat [25]. It is not due to a deiodinating microsomal system activation.

It can be observed from Table 2 that dexamethasone lowered cytochrome P450 content in liver and adrenal cortex microsomes and in adrenal cortex mitochondria. If this effect in adrenal cortex could be explained by the inhibition of ACTH secretion, the physiological significance of the liver microsomal cytochrome P450 findings remains to be clarified. The results of our experiments confirm that phenobarbital significantly increased the cytochrome P450 content in liver microsomes, but had no effect on adrenal cor-

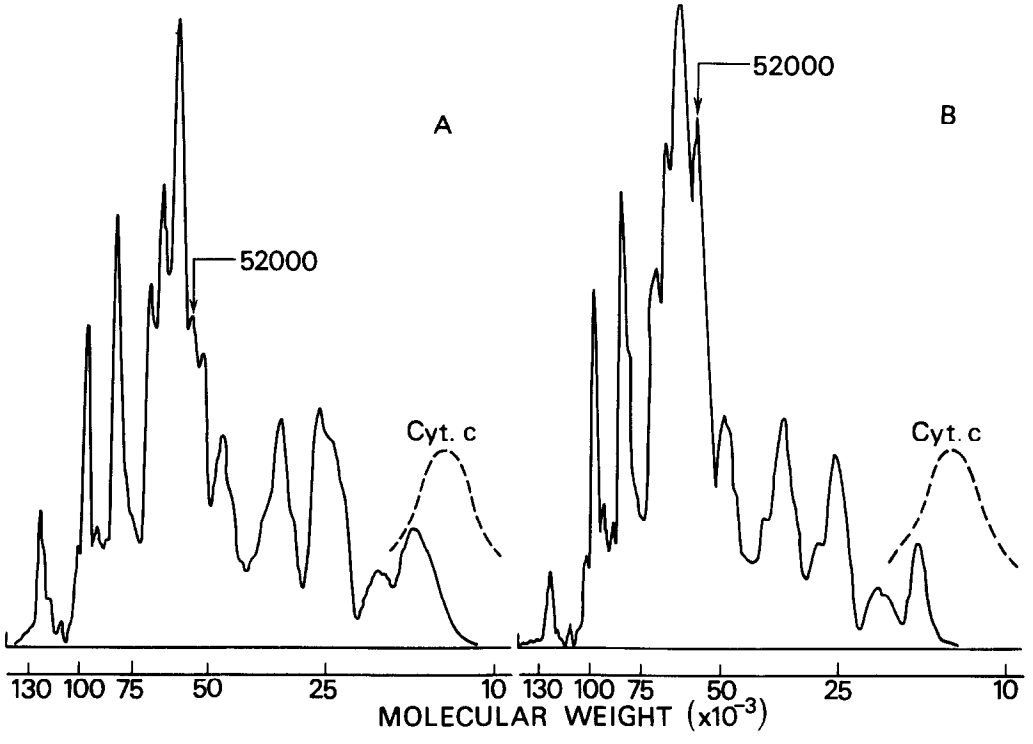

Fig. 1. Densitometric traces of polyacrylamide gel electrophoresis of liver microsomal proteins. (A) Normal, (B) phenobarbital. Cytochrome *c*, represented by broken lines, was used as a marker.

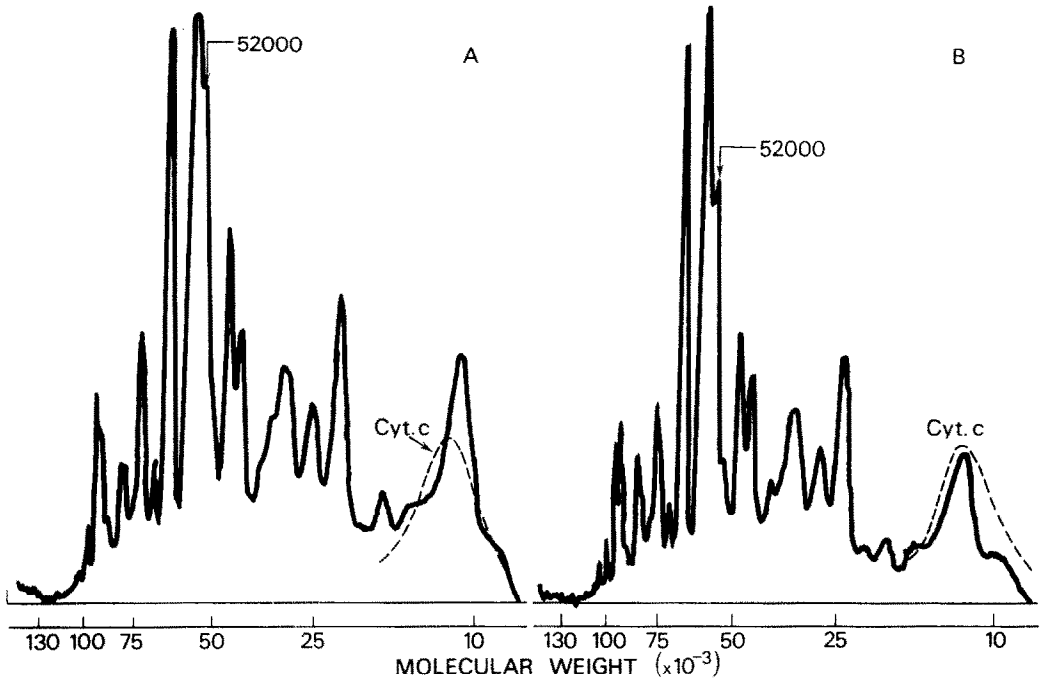

Fig. 2. Densitometric traces of polyacrylamide gel electrophoresis of adrenal cortex mitochondria. (A) Normal, (B) dexamethasone.

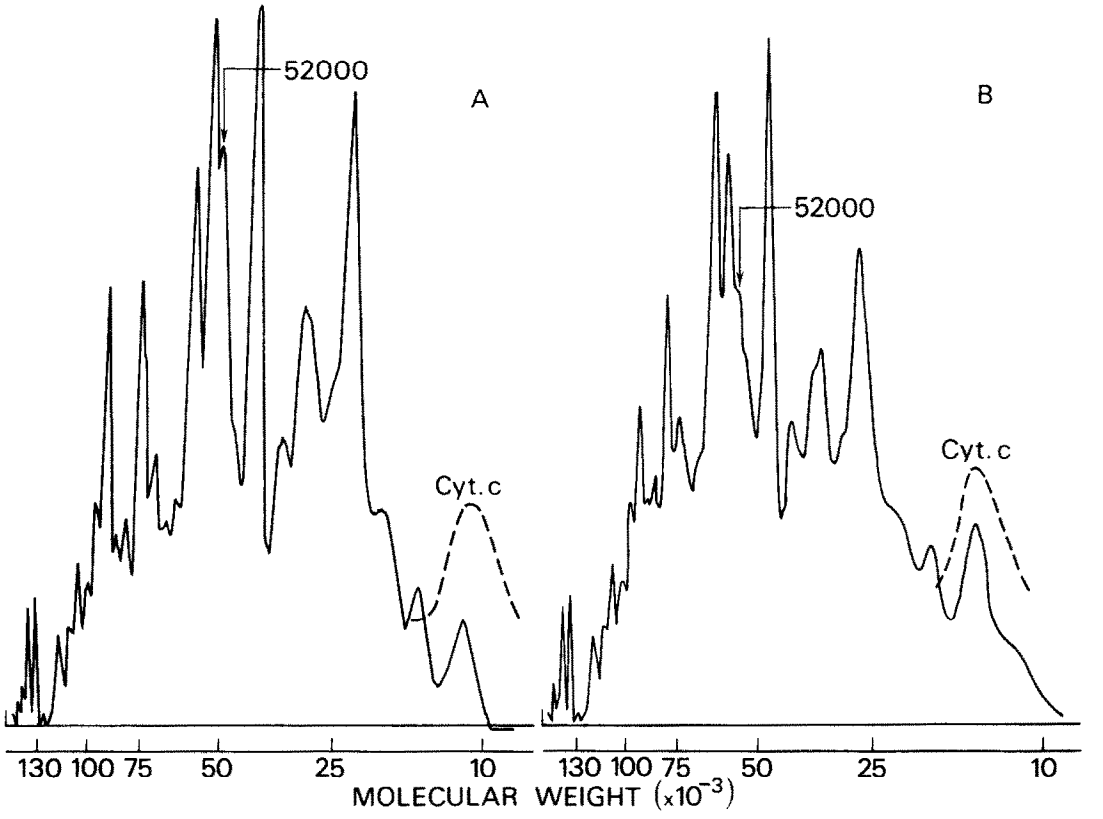

Fig. 3. Densitometric traces of polyacrylamide gel electrophoresis of adrenal cortex microsomes. (A) Normal, (B) dexamethasone.

tex cytochrome P450 content. These findings strongly suggest that phenobarbital has no inducing activity on adrenocortical cytochrome P450.

Polyacrylamide gel electrophoresis revealed that the amount of the protein constituent with a molecular mass of 52,000 dalton, which contains cytochrome P450 subunits [26] doubled in liver microsomal proteins from phenobarbital treated rats (Fig. 1). No change of this protein concentration was observed in dexamethasone treated rats. This is in accordance with the previous spectrophotometric results of cytochrome P450 concentration. This protein fraction was decreased by 20% and 28% respectively in adrenal cortex mitochondria and microsomes after dexamethasone treatment (Figs 2 and 3). It was not affected by phenobarbital treatment.

In conclusion, the effects of phenobarbital and dexamethasone treatments appear to induce changes in adrenal cortex and liver cytochrome P450 contents, and serum iodothyronines concentrations by different and independent mechanisms.

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