The Role of Glucocorticoids in the Regulation of the Diurnal Rhythm of Hepatic β -Hydroxy- β -methylglutaryl-Coenzyme A Reductase and Cholesterol 7 α -Hydroxylase

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The microsomal activities of the hepatic enzymes hydroxymethylglutaryl-CoA reductase and cholesterol 7 α -hydroxylase exhibit a diurnal rhythm with maximum activities observed during the dark period and minimum activities around noon (12:00h). This diurnal rhythm was maintained for both enzymes after adrenalectomy, but the amplitude of variation for the activity of both enzymes was greatly decreased. A single injection of cortisol administered to adrenalectomized rats 3h before the expected maximum in enzyme activity resulted in a twofold increase in the activity of both enzymes 3h later, at values similar to those observed for control rats killed at the same time. This response appeared to require protein synthesis, since it was blocked by actinomycin D. However, the administration of cortisol to adrenalectomized rats 3h before the expected minimum did not result in significant change in the activity of hydroxymethylglutaryl-CoA reductase and cholesterol 7 α -hydroxylase 3h later. Kinetic studies of cholic acid metabolism *in vivo* demonstrated that adrenalectomy results in a significant decrease in the rate of synthesis of cholic acid and a considerable decrease in the pool size of cholic acid and its metabolic products. Treatment of adrenalectomized rats with cortisol increased the rate of synthesis of cholic acid and restored the pool size. These results are consistent with the effects of adrenalectomy and cortisol treatment on the activity of cholesterol 7 α -hydroxylase.

The metabolism of cholesterol in the liver is subject to complex control and is altered by a number of environmental, hormonal and dietary factors. In the adult rat, maintenance of a constant amount of cholesterol in the body seems to be achieved largely by regulation of hepatic cholesterologenesis and of the conversion of cholesterol into bile acids. Although it is now generally accepted that the two processes are independently controlled, the fact that changes in the rate of synthesis of cholesterol are in many cases accompanied by parallel changes in the rate of bile-acid biosynthesis suggests that some of the physiological mechanisms concerned in the regulation of cholesterologenesis and catabolism of cholesterol must be common. Several factors that influence the rate of hepatic cholesterol synthesis are known to do so by modifying the activity of the microsomal enzyme hydroxymethylglutaryl-CoA reductase (White & Rudney, 1970). Factors that influence the rate of synthesis of bile acids have noticeable parallel effects on the activity of cholesterol 7 α -hydroxylase (Mitropoulos *et al.*, 1973). The activities of both these enzymes vary diurnally, with maximum activities observed during the middle of the dark period and minimum around noon, probably owing to an alternating cycle of repression and de-repression

of enzyme synthesis (Higgins *et al.*, 1971; Gielen *et al.*, 1969; Mitropoulos *et al.*, 1972).

In the present paper we attempt to investigate some aspects of the physiological mechanism concerned in the regulation of the diurnal rhythm of hydroxymethylglutaryl-CoA reductase and of cholesterol 7 α -hydroxylase by studying the effects of adrenalectomy and glucocorticoid replacement on the diurnal rhythm of the activity of these enzymes. Because previous experimental evidence for the role of glucocorticoids on the diurnal rhythm of hydroxymethylglutaryl-CoA reductase is inconsistent, we have studied at the same time the response of the soluble enzyme tyrosine transaminase, an enzyme whose regulation is better understood (Wurtman, 1974).

Materials and Methods

Materials

[4-¹⁴C]Cholesterol (60mCi/mmol), [24-¹⁴C]cholic acid (51mCi/mmol) and DL-[2-³H]mevalonic acid lactone (235mCi/mmol) were obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). DL-3-Hydroxy-3-methyl[3-¹⁴C]glutaric acid (15.8mCi/mmol) was obtained from NEN Chemicals

G.m.b.H. (Dreieichenhain, Germany). Radioactively labelled cholesterol was purified before use by t.l.c. on Kieselgel H at 5°C with diethyl ether as solvent. 7 α -Hydroxy[4-¹⁴C]cholesterol was synthesized as described previously (Mitropoulos & Balasubramaniam, 1972). Cholesterol and cholic acid were obtained from commercial sources and were crystallized three times before use. Methyl cholate was prepared as previously described (Mitropoulos & Myant, 1967). The sources of other animals used in this work have been reported elsewhere (Balasubramaniam *et al.*, 1973).

Housing and treatment of rats

Male rats of an inbred Wistar strain weighing initially 110–120 g were kept for 2 weeks before each experiment under conditions of controlled lighting and feeding in a room maintained at 24°C. Fluorescent lighting was switched on between 9:00 and 19:00 h; between 19:00 and 9:00 h the animals were kept in complete darkness. Food, given *ad libitum*, was provided only during the dark period. Drinking water was provided throughout the whole 24 h cycle. At the end of 2 weeks, bilateral adrenalectomy was performed on some of the rats and the rest were sham-operated to provide the adrenalectomy controls. Adrenalectomized rats had NaCl (1%, w/v) in their drinking water. At 10–14 days after adrenalectomy, the rats were killed at the designated time, the livers were removed and subcellular fractions were prepared for the assay of enzymes. For some experiments adrenalectomized rats were injected intraperitoneally with glucocorticoids or adrenalin solution, whereas corresponding controls received injections of the hormone solvent. All rats were fed on commercial pellets of a composition described previously (Mitropoulos *et al.*, 1973).

For kinetic studies on the turnover of cholic acid, adrenalectomized and sham-operated rats received an intraperitoneal injection of [24-¹⁴C]cholic acid (6 μ Ci/rat, 119 μ Ci/mg) on the tenth day after adrenalectomy. Adrenalectomized rats treated with cortisol (500 μ g/rat per day) received a cortisol injection 24 h before they received [¹⁴C]cholic acid and daily injections of cortisol for 7 days thereafter, at 19:00 h. All the rats were housed in individual cages and the faeces were collected daily for 7 days. At the end of the seventh day the rats were killed, and the intestine and caecum and their contents, together with the liver, were removed and stored in the deep-freeze.

Pool size of cholic acid and its metabolites

The intestine and caecum and their contents and the liver from each rat were minced and transferred into a round-bottom flask with 150 ml of ethanol, and

the mixture was refluxed for 2 h. The ethanol extracts were removed, 150 ml of chloroform/methanol (2:1, v/v) were added, and the mixture was refluxed again for 2 h. The process was repeated once more. The combined extracts were evaporated to dryness and dissolved in ethanol, and a portion was removed for assay of radioactivity. Another portion was removed to measure the specific radioactivity of cholic acid. This portion was subjected to hydrolysis in aq. 1 M-NaOH for 3 h at 120°C. After removal of the non-saponifiable fraction with ether, the aqueous phase was acidified and extracted with ether. The ether extract containing free bile acids was subjected to t.l.c. with chloroform/methanol (7:3, v/v) as the solvent. The band of radioactivity containing cholic acid was located by radioautography and eluted, and cholic acid was methylated. The methyl cholate fraction was further purified on t.l.c. with acetic acid/di-isopropyl ether/iso-octane (1:1:2, by vol.). The radioactive band containing methyl cholate was located by radioautography and eluted. Portions were taken for determination of the total amount of methyl cholate present and for its specific radioactivity. Methyl cholate was quantitatively determined by g.l.c. An appropriate volume, containing 0.5–2 μ g of methyl cholate, was injected directly on to a column of a Varian model 2740 gas chromatograph with flame-ionization detector. The stationary phase was either 1.5% SE-30 or 3% QF1 supported on Varaport (80–100 mesh). The column temperature was 260°C, the detector temperature 270°C and the injector temperature 270°C. The carrier gas was N₂ at a flow rate of 40 ml/min for the QF1 column and 50 ml/min for the SE-30 column. Under the operating conditions used, methyl cholate was the only peak present and the height of the peak was proportional (over the range used) to the methyl cholate injected.

The pool of cholic acid and its metabolic products was thus estimated from the total radioactivity present in the pools of bile acids and the specific radioactivity of the cholic acid, assuming that at the time of death the specific radioactivity of various metabolic products of cholic acid is equal to that of their precursor.

Cholate half-life and synthesis

Cholic acid half-life and fractional turnover rate were determined by the method of Lindstedt & Norman (1956). Faecal samples were transferred to round-bottom flasks with 150 ml of ethanol. The mixture was refluxed for 2 h. The ethanol extract was removed and the dry residue was powdered. This powder was refluxed with 150 ml of chloroform/methanol (2:1, v/v), the organic extracts were removed and the process was repeated once more. The combined extracts were evaporated, the residue

was dissolved in ethanol and portions were removed for assay of radioactivity. For all rats the total radioactivity excreted with the faeces plus the radioactivity in the bile acid pools was not less than 94% of the injected radioactivity. Cholate synthesis was calculated from the pool size of cholate and its metabolites and the fractional turnover rate of ^{14}C radioactivity.

Assay of liver enzymes

The preparation of subcellular fractions, incubation conditions and assay of 7α -hydroxycholesterol formed during the incubation were described previously (Mitropoulos & Balasubramaniam, 1972; Balasubramaniam *et al.*, 1973).

Hydroxymethyl[3- ^{14}C]glutaryl-CoA was prepared from the β -hydroxy- β -methyl[3- ^{14}C]glutaric acid by the method of Louw *et al.* (1969). The activity of hydroxymethylglutaryl-CoA reductase was measured in portions of microsomal preparations containing no more than 2mg of microsomal protein. The incubation mixture contained, in a total volume of 2ml: the microsomal preparation, 0.1M-potassium phosphate buffer (pH7.4), 5mM-MgCl₂, 30mM-nicotinamide, 50mM-glutathione, 25mM-glucose 6-phosphate, 2.5mM-NADP⁺, 1 unit of glucose 6-phosphate dehydrogenase activity and 0.18mM-DL-hydroxymethyl[3- ^{14}C]glutaryl-CoA (342 $\mu\text{Ci}/\text{mmol}$). This mixture was incubated for 30min at 37°C. Under these conditions, enzyme activity was linear with respect to time and to protein concentration (in the range used). Each assay was performed in triplicate and the results were averaged. The enzymic reaction was stopped by the addition of 1ml of 0.5M-H₂SO₄ and 25 μmol of DL-[2- ^3H]mevalonic acid (1.8 $\mu\text{Ci}/\text{mmol}$). The mixture was incubated for 30min at 37°C to ensure lactonization of the mevalonate. The rate of incorporation was determined after t.l.c. of the product by using the [^3H]mevalonic acid lactone to correct for recovery (Goldfarb & Pitot, 1971).

The activity of tyrosine transaminase in the soluble fraction of liver homogenates was assayed by the method of Diamondstone (1966).

Other assays

For measurement of cholesterol content in serum, liver homogenate and microsomal fraction, sterols were first extracted by the method of Folch *et al.* (1957) and cholesterol was determined in a portion of the extract by the g.l.c. method of Ishikawa *et al.* (1974).

Corticosterone in serum samples was measured by the fluorimetric method of Gibbs (1970). Protein was assayed by the method of Cleland & Slater (1953).

Results

Effect of adrenalectomy on the activities of hydroxymethylglutaryl-CoA reductase, cholesterol 7α -hydroxylase and tyrosine transaminase

Rats that had bilateral adrenalectomy 10 days before they were killed at 7:00h had serum corticosterone concentrations of 4.7 $\mu\text{g}/100\text{ml}$ (Table 1), whereas sham-operated animals killed at the same time had concentrations of 11.2–31.2 $\mu\text{g}/100\text{ml}$. These results are comparable with those experienced in other laboratories (Scheving & Pauly, 1966; Cheifetz *et al.*, 1968), and suggest lack of circulating corticosterone in the adrenalectomized rats. After the first post-operative week, adrenalectomized rats consumed amounts of food similar to those consumed by the sham-operated controls and both groups gained weight similarly. Serum cholesterol concentrations in adrenalectomized rats were not significantly different from those observed in the controls (Table 1). However, the cholesterol content (free plus esterified) of microsomal preparations from livers of adrenalectomized rats was consistently higher than that of the sham-operated controls.

Fig. 1 shows the activity of hydroxymethylglutaryl-CoA reductase, cholesterol 7α -hydroxylase and tyrosine transaminase in liver subcellular fractions from sham-operated and adrenalectomized rats killed at various times during the diurnal cycle. These results show that the activity of hydroxymethylglutaryl-CoA reductase and of cholesterol 7α -hydroxylase retains a diurnal rhythm after bilateral

Table 1. *Effect of adrenalectomy on liver microsomal cholesterol content and serum corticosterone and cholesterol concentrations*

The rats were adapted to controlled feeding and lighting for 14 days before adrenalectomy or sham operation. At 10 days after the operation the rats were killed at 07:00h, within a short period, by guillotine decapitation. Blood was collected to prepare the serum and the livers were removed to prepare the microsomal fraction. There were five animals in each group, and mean values $\pm$ S.D. are given. Other experimental details are given in the Materials and Methods section.

Treatment	Serum [corticosterone] ($\mu\text{g}/100\text{ml}$)	Serum [cholesterol] (mg/100ml)	Microsomal [cholesterol] (nmol/mg protein)
Control	24.2 $\pm$ 8.0	44.6 $\pm$ 4.9	46.0 $\pm$ 5.7
Adrenalectomy	4.7 $\pm$ 0.7	43.6 $\pm$ 6.1	59.9 $\pm$ 6.1

Fig. 1. Diurnal rhythms in the activity of (a) hydroxymethylglutaryl-CoA reductase, (b) cholesterol 7 α -hydroxylase and (c) tyrosine transaminase in the liver of adrenalectomized and sham-operated rats

The rats were adapted to controlled lighting and feeding for 2 weeks before they were adrenalectomized or sham-operated. At 10–15 days after the operation, groups of rats were killed at the times designated, and the livers were removed to prepare subcellular fractions. Each group contained at least three rats and the results are given as means $\pm$ S.D. (vertical bar). ○, Adrenalectomized; ●, sham-operated. Other experimental details are given in the Materials and Methods section.

adrenalectomy. In both cases the amplitude of variation is considerably decreased, since the decrease in the activity of the enzymes after adrenalectomy is much greater during the dark period than that around noon. In agreement with results of other workers (Wurtman & Axelrod, 1967; Wurtman, 1974), the effects of adrenalectomy on the diurnal rhythm of tyrosine transaminase were minor (Fig. 1).

Glucocorticoid replacement in adrenalectomized rats and the restoration of normal diurnal rhythm

Since adrenalectomy resulted in measurable effects on the activities of hydroxymethylglutaryl-CoA reductase and cholesterol 7 α -hydroxylase, hormone-replacement experiments were performed.

Intraperitoneal administration of adrenaline (100 μ g/100 g body wt. of rat) to adrenalectomized rats either during the day or at night resulted in no increase in hydroxymethylglutaryl-CoA reductase and cholesterol 7 α -hydroxylase activities 3 h later. This is in complete agreement with the results of Edwards (1973) on the effect of a single injection of adrenaline on the activity of hydroxymethylglutaryl-CoA reductase in adrenalectomized rats. Preliminary experiments showed that daily injection of cortisone (500 μ g/100 g body wt. of rat) for 6 days to adrenalectomized rats restored the activities of both hydroxymethylglutaryl-CoA reductase and cholesterol 7 α -hydroxylase to values, at midday and at midnight (00:00h), similar to those observed for sham-

operated animals injected daily with hormone solvent and killed at the same time.

To see the effect of a single injection of glucocorticoids on the activities of the enzymes, adrenalectomized rats were injected with cortisol phosphate (250 μ g/100 g body wt. of rat) either at 10:00h or at 22:00h, and were killed 3 h later, either at 13:00h or at 01:00h. Groups of sham-operated controls and adrenalectomized rats were killed at the same time after having received an injection of saline 3 h earlier. The activities of hydroxymethylglutaryl-CoA reductase and cholesterol 7 α -hydroxylase in adrenalectomized rats killed at 01:00h were lower than the activities of the corresponding sham-operated controls, and they increased by about twofold in the adrenalectomized rats that received cortisol (Table 2). No significant effects of cortisol on the activities of hydroxymethylglutaryl-CoA reductase and of cholesterol 7 α -hydroxylase were obtained when the hormone was injected into adrenalectomized rats at 10:00h and the rats were killed 3 h later. The activity of tyrosine transaminase at 01:00h in adrenalectomized rats that received cortisol was similar to that in the sham-operated controls, whereas the activity of the enzyme at 13:00h in the same group of rats was six times higher than that in the corresponding sham-operated controls.

To ascertain whether the cortisol-induced increase in the activity of hydroxymethylglutaryl-CoA reductase, cholesterol 7 α -hydroxylase and tyrosine trans-

Table 2. *Effect of adrenalectomy and cortisol treatment on the activity of hepatic hydroxymethylglutaryl-CoA reductase, cholesterol 7 α -hydroxylase and tyrosine transaminase*

The rats were adapted to controlled feeding and lighting for 14 days before adrenalectomy or sham operation. Adrenalectomized rats received a single intraperitoneal injection of a solution of cortisol phosphate (250 μ g/100g body wt. of rat) in saline, either at 10:00h or at 22:00h. Sham-operated controls and adrenalectomized rats received injections of saline at the same time. All rats were killed 3h after the injection, either at 01:00h or at 13:00h. There were three animals in each group, and mean values $\pm$ S.D. are given. Other experimental details are given in the Materials and Methods section.

Treatment	Hydroxymethylglutaryl-CoA reductase (pmol/min per mg of protein)		Cholesterol 7 α -hydroxylase (pmol/min per mg of protein)		Tyrosine transaminase (nmol/min per mg of protein)	
	13:00h	01:00h	13:00h	01:00h	13:00h	01:00h
Control	87 $\pm$ 19	492 $\pm$ 79	25.2 $\pm$ 4.5	48.0 $\pm$ 3.1	8.0 $\pm$ 3.1	57.8 $\pm$ 3.7
Adrenalectomized	70 $\pm$ 17	168 $\pm$ 15	17.5 $\pm$ 2.5	28.0 $\pm$ 5.5	6.7 $\pm$ 1.0	36.6 $\pm$ 8.2
Adrenalectomized + cortisol	54 $\pm$ 10	387 $\pm$ 65	25.5 $\pm$ 6.5	44.5 $\pm$ 7.5	41.2 $\pm$ 0.8	60.6 $\pm$ 13.3

Table 3. *Effect of adrenalectomy and cortisol treatment on the pool size and the daily synthesis of cholic acid*

The rats were adapted to controlled feeding and lighting for 14 days before adrenalectomy or sham operation. Each group contained five rats that were housed in individual cages. All rats received an intraperitoneal injection of [24- 14 C]cholic acid on the tenth day after the operation. Adrenalectomized rats treated with cortisol received a cortisol injection 24h before they received [14 C]cholic acid and daily injections (at 20:00h) for 7 days thereafter. At the end of the seventh day all rats were killed. Values are expressed as means $\pm$ S.D. for the five rats in each group. Other experimental details are given in the Materials and Methods section.

Treatment	Weight of animals at end of experiment (g)	Pool size (μ mol/100g body wt. of rat)	Half-life (days)	Rate of synthesis (μ mol/day per 100g body wt. of rat)
Control	235 $\pm$ 9	20.2 $\pm$ 1.7	2.15 $\pm$ 0.17	6.6 $\pm$ 0.9
Adrenalectomy	197 $\pm$ 39	11.2 $\pm$ 3.1	2.22 $\pm$ 0.42	3.5 $\pm$ 1.1
Adrenalectomy + cortisol	200 $\pm$ 8	18.8 $\pm$ 3.0	2.63 $\pm$ 0.31	5.3 $\pm$ 0.6

aminase involved protein synthesis, adrenalectomized rats were injected with cortisol and actinomycin D simultaneously at 20:00h, and the rats were killed 3h later. The activity of the enzymes was compared with that in adrenalectomized rats that received cortisol only, adrenalectomized rats that received saline, and sham-operated controls that received saline and were killed 3h later at the same time. Actinomycin D blocks the cortisol-induced increase in the activities of hydroxymethylglutaryl-CoA reductase, cholesterol 7 α -hydroxylase and tyrosine transaminase.

Influence of glucocorticoids on the rate of synthesis, pool size and turnover rate of cholic acid

The effects of adrenalectomy and glucocorticoid replacement on the activity of cholesterol 7 α -hydroxylase observed *in vitro* suggest measurable effects on the overall rate of synthesis of cholic acid under those conditions. We have, therefore, compared certain parameters related to the metabolism of cholic acid in adrenalectomized rats, adrenalectomized-cortisol-treated rats and sham-operated controls. There was a 45% decrease in pool size of cholate and its metabolites in adrenalectomized rats,

as compared with the sham-operated controls, but this pool size in cortisol-treated adrenalectomized rats was not significantly different from that of the sham-operated controls (Table 3). Faecal-elimination curves after the administration of [14 C]cholate yielded first-order kinetics in all groups of rats, suggesting that during the time of study (10–20 days after the operation) cholate metabolism was in a relatively steady state. There was no considerable difference, in the half-life and fractional turnover rate of cholic acid, between adrenalectomized rats, adrenalectomized-cortisol-treated rats and sham-operated controls, resulting in a daily rate of synthesis for cholic acid in adrenalectomized rats that was significantly lower than that in the sham-operated controls (Table 3). Adrenalectomized rats that had daily injections of cortisol during the experiment had a rate of synthesis of cholic acid approaching that observed in the control animals.

Discussion

The present results show that the diurnal rhythm in the activity of hydroxymethylglutaryl-CoA reductase and of cholesterol 7 α -hydroxylase was maintained

after adrenalectomy. However, as the decrease in the activity of the enzymes in adrenalectomized rats, compared with the activity in the corresponding controls, was much greater during the dark period than during the light period, the amplitude of variation for the activity of both enzymes was considerably decreased.

Previous results on the effect of adrenalectomy on the diurnal rhythm of the hydroxymethylglutaryl-CoA reductase activity are contradictory. Huber *et al.* (1972) found that adrenalectomy had no effect on the diurnal rhythm of the hydroxymethylglutaryl-CoA reductase, whereas Edwards (1973) has reported that adrenalectomy abolished this variation, with low enzyme activity approximating to that of the intact rats killed at noon. The present results are consistent with the latter report (Edwards, 1973) in so far as adrenalectomy results in a decreased enzyme activity to a greater extent during the dark period than during the light period of the light cycle. With regard to cholesterol 7 α -hydroxylase, the previous findings are less contradictory. Gielen *et al.* (1970) and Van Cantfort (1973) have reported loss of diurnal rhythm in the activity of this enzyme in rats that have been adrenalectomized or had decreased amounts of circulating corticosterone. Under both the above conditions, enzyme activity remained throughout the diurnal cycle at the minimal value observed in the control rats at about noon. The present results agree with the above reports, although under our conditions adrenalectomy does not completely abolish the diurnal rhythm in cholesterol 7 α -hydroxylase activity. Our results on the effect of adrenalectomy on tyrosine transaminase are clearly consistent with the previous findings of Wurtman & Axelrod (1967).

Since serum corticosterone concentrations in the rat are known to undergo a diurnal variation, with peak values being reached at the onset of the dark period (Scheving & Pauly, 1966; Cheifetz *et al.*, 1968), it is reasonable to consider the importance of this hormone in the regulation of the diurnal rhythm of the activity of hydroxymethylglutaryl-CoA reductase and of cholesterol 7 α -hydroxylase. Our results on glucocorticoid replacement confirm that circulating corticosterone is an important factor for the maintenance of the amplitude of the diurnal variation in the activities of hydroxymethylglutaryl-CoA reductase and cholesterol 7 α -hydroxylase. Moreover, since a single injection of cortisol into adrenalectomized rats at the beginning of the dark period restored the peak activity of hydroxymethylglutaryl-CoA reductase and of cholesterol 7 α -hydroxylase to the values observed for control animals, and since these effects were blocked by actinomycin D, it is reasonable to assume that glucocorticoids are responsible for the induction of synthesis of hydroxymethylglutaryl-CoA reductase

and cholesterol 7 α -hydroxylase that occurs in the early part of the dark period. However, the fact that injection of cortisol into adrenalectomized rats at 10:00h did not affect the activity of the enzyme 3h later suggests that glucocorticoids are not the only factor triggering the induction of hydroxymethylglutaryl-CoA reductase and cholesterol 7 α -hydroxylase. By contrast, cortisol injection into adrenalectomized rats resulted in increased activity of tyrosine transaminase to values that were similar in both the light and the dark phases of the light cycle.

The present results show that adrenalectomy results in a significant decrease in the rate of synthesis of cholic acid, and that treatment with cortisol restored the rate of synthesis of the bile acid. Since the rate of formation of 7 α -hydroxycholesterol from cholesterol is generally assumed to control the rate of synthesis of cholic acid, the results *in vitro* on the effect of adrenalectomy and glucocorticoid replacement on the activity of cholesterol 7 α -hydroxylase are consistent with the effects observed *in vivo* on the kinetics of cholic acid metabolism under the same conditions.

In the rat, maintenance of a constant amount of cholesterol in the body is achieved largely by the regulation of hepatic cholesterol synthesis and conversion into bile acids. Adrenalectomy results in a decreased rate of synthesis of cholic acid, the most important bile acid in the rat, and in an overall decreased rate of formation of 7 α -hydroxycholesterol from cholesterol. One of the major compartments of exchangeable cholesterol in the body is the serum cholesterol, and present and previous results (Nervi & Dietschy, 1974) show that serum cholesterol concentrations do not change significantly after adrenalectomy. It is therefore reasonable to postulate that a decreased rate of conversion of cholesterol to the major bile acid will be compensated for by a decreased overall rate of synthesis of hepatic cholesterol. The present results show that effects of adrenalectomy and glucocorticoid replacement on the activity of cholesterol 7 α -hydroxylase are accompanied by parallel effects on the activity of hydroxymethylglutaryl-CoA reductase, the enzyme that controls the rate of hepatic cholesterologenesis.

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