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The Circadian Rhythm of Synthesis and Catabolism of Cholesterol*

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Abstract. The circadian rhythms of HMG-CoA reductase and cholesterol-7α-hydroxylase (low values during light, rising in the evening with maximum at 12.00 p.m.) are investigated in rats under diverse conditions.

Intragastral administration of cholestyramine (bile acid-absorbing resin) leads to an increased rhythm of both enzymes. Feeding of cholic acid (or cholesterol) reduces the activity of both enzymes (of HMG-CoA reductase and cholesterol-7α-hydroxylase, respectively). In starved rats enzyme activities are lowered, too; a damped rhythm reappears after 24 h. A 20% fat diet (containing saturated fatty acids predominantly) markedly reduces the high values.

Enzyme activities inhibited after thyroidectomy can be normalized by thyroxin substitution. Thyroxin administration in the normal remains without effect. Four-day insulin treatment of the normal inhibits cholesterol-7α-hydroxylase, has no effect on HMG-CoA reductase. In the untreated diabetic rat cholesterol-7α-hydroxylase is increased, HMG-CoA reductase significantly inhibited. Insulin treatment of the diabetic animal results in normalized values of HMG-CoA reductase whilst cholesterol-7α-hydroxylase is nearly completely suppressed.

The rate-limiting enzymes of cholesterol turnover are peripherally regulated by their products via a negative feedback. In contrast, hormones may have synergistic or opposite effects; thus they may represent means of higher regulation. All regulative possibilities discussed (except hypophysectomy) do modify the circadian rhythms. This cannot be demonstrated after hypophysectomy. After hypophysectomy circadian rhythms are not detectable any more.

To get valid data about biochemical or pharmacological effects on these enzymes the circadian variations have to be considered by measuring at different times of day (e.g. fat diet); for only the area of enzyme activity and time of day is proportional to the metabolism of a substrate.

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Key words: Circadian rhythm of HMG-CoA reductase and cholesterol-7α-hydroxylase – Influences of starvation – of cholesterol – Bile acid and cholestyramine feeding – Thyroxin – Insulin – Hypophysectomy.

Zusammenfassung. Der Circadianrhythmus von HMG-CoA Reduktase und Cholesterin-7α-hydroxylase (niedrige Werte am Tage, ansteigende in der Nacht mit Maximum um 24.00 Uhr) wird unter verschiedenen Bedingungen an Ratten untersucht.

Intragastrale Applikation von Cholestyramine (Bindung von Gallensäuren im Darm) führt zu erhöhten Enzymaktivitäten unter Vergrößerung des Rhythmus'. Cholsäure-Fütterung hemmt beide Enzymaktivitäten (Cholesterin-Zufuhr hemmt die HMG-CoA Reduktase). Im Hungerzustand entwickelt sich nach 24 Std ein gedämpfter Rhythmus bei beiden Enzymen. Eine 20%ige Fett-Diät (vorwiegend gesättigte Fettsäuren) senkt die Enzymaktivität durch Reduzierung der Maximalwerte bei nahezu unveränderten Niedrigwerten.

Die nach Thyreoidektomie erniedrigten Enzymaktivitäten können durch Hormonsubstitution normalisiert werden. Experimentell erhöhte Hormonspiegel bleiben ohne Effekt. Insulinbehandlung des Normaltieres hemmt die Cholesterin-7α-hydroxylase, beeinflußt die HMG-CoA Reduktase nicht. Im diabetischen Tier zeigt sich umgekehrt eine erhöhte Cholesterin-7α-hydroxylase und eine gehemmte HMG-CoA Reduktase Aktivität. Insulinbehandlung dieser Tiere normalisiert die HMG-CoA Reduktase, während die Cholesterin-7α-hydroxylase gehemmt wird.

Die geschwindigkeitsbestimmenden Enzyme des Cholesterin-Stoffwechsels werden peripher reguliert durch ihre Produkte im Sinne einer negativen feedback-Koppelung. Im Gegensatz dazu können Hormone gleich- und gegensinnige Effekte auf die beiden Enzyme ausüben und so Mittel einer übergeordneten Steuerung darstellen. Alle besprochenen Regulationsmöglichkeiten wirken modifizierend auf den Circadianrhythmus mit Ausnahme der Hypophysektomie. Nach Entfernen der Hypophyse ist der Circadianrhythmus nicht mehr nachweisbar.

Um gültige Aussagen über biochemische oder pharmakologische Wirkungen auf diese Enzyme machen zu können, muß der circadiane Rhythmus durch Messungen zu mehreren Tageszeiten berücksichtigt werden (Beispiel Fett-Diät); denn nur die Fläche aus Enzymaktivität und Tageszeit ist proportional dem Umsatz einer Substanz.

This paper deals with the circadian rhythm of cholesterol biosynthesis and catabolism, the influence of the products of cholesterol and bile acids and of hormones on this rhythm. A review of the investigations of other authors is included.

Cholesterol turnover in the liver cell depends on cholesterol uptake with food, on cholesterol biosynthesis, and cellular catabolism and output. Biosynthesis and catabolism of cholesterol show distinct circadian variations caused by corresponding oscillations of the rate-limiting enzymes of both metabolic pathways. The formation of mevalonate from hydroxymethylglutaryl-coenzyme A (Siperstein, 1960) is the rate-limiting step of the de novo synthesis of cholesterol from acetate. It is catalysed by

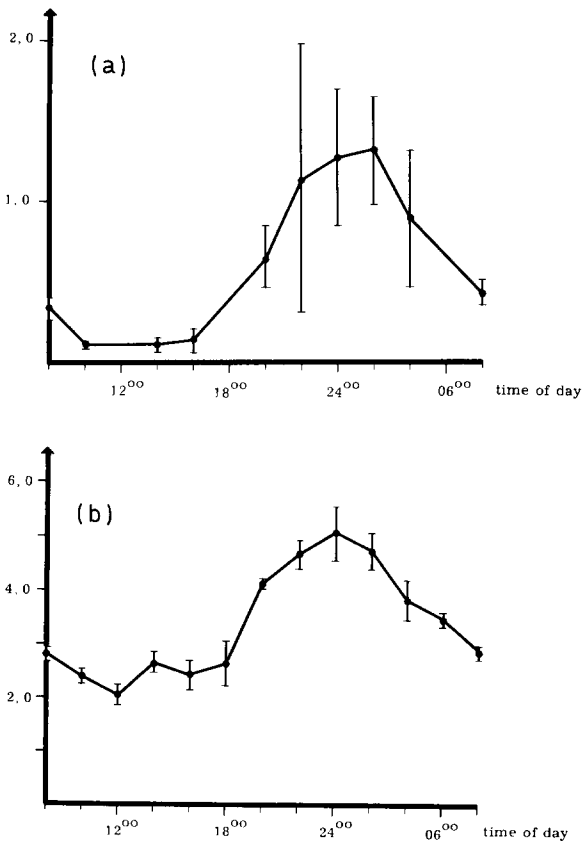

Fig. 1. **a** Course of HMG-CoA reductase activity [μ mol/g liver/h] in untreated rats; food and water ad lib.; 4 animals per point (light 07.00–19.00; dark 19.00–07.00) (reproduced from Hamprecht, 1969). **b** Course of cholesterol-7α-hydroxylase activity (rel) $\pm$ $s\bar{x}$ in untreated rats; food and water ad lib.; 5 rats per point (light 07.00–19.00; dark 19.00–07.00)

hydroxymethylglutaryl-coenzyme A reductase (HMG-CoA reductase). The rate-limiting step of cholesterol catabolism is the formation of 7α-hydroxycholesterol from cholesterol catalysed by cholesterol-7α-hydroxylase (Danielsson, 1967; Shefer, 1969). The investigations reported here deal with these two enzymes. Both metabolic pathways are regulated by their products which have a negative feedback effect on the rate-limiting enzymes. Cholesterol and bile acids inhibit their own synthesis. In addition, hormonal influences are investigated.

All experiments were carried out with rats. These night-active animals, which have their eating phase with the beginning of darkness, lived in an artificial light-dark rhythm (dark phase: 19.00–07.00 h). They were fed a standard diet for rats (Altromin C 1000[1]) and given water ad lib. unless otherwise noted.

The first investigations on the rhythm of HMG-CoA reductase (Hamprecht) and of cholesterol-7α-hydroxylase (Gielen) were published in 1969. Independently, we

[1] Altromin, Lage/Lippe

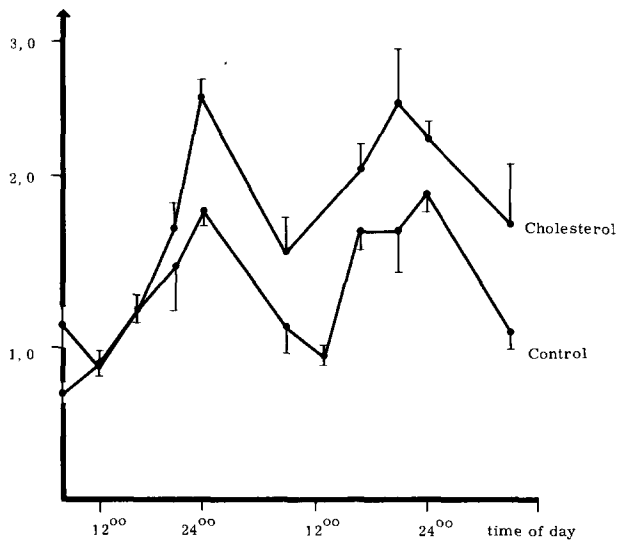

Fig. 2. Course of cholesterol-7α-hydroxylase activity (rel) $\pm s\bar{x}$ in untreated (controls) and in cholestyramine-treated rats; food and water ad lib.; 5 animals per point. *Cholestyramine*: 85 mg/100 g body weight via intragastric tube, administration every 12 h, first 1 h before experiment (light 07.00–19.00; dark 19.00–07.00)

reported on the circadian rhythm of cholesterol-7α-hydroxylase activity with a different method at the same time (Mayer, 1972a).

Figure 1 shows the circadian rhythm of both enzymes in the normal rat. They have a minimum during the light period; at the beginning of the dark period they increase and reach a maximum at about 12.00 p.m.

The following investigations are based on the questions: How do substances which have an effect on enzyme activity influence the circadian rhythm? Is it possible to eliminate the rhythm?

Since the product inhibition was well known we began by investigating the circadian rhythms after artificial addition and removal of cholesterol or bile acids, respectively. By oral administration of cholestyramine the content of bile acids in the enterohepatic circulation is lowered. Cholestyramine is a non-absorbable resin which binds bile acids in the intestine. A significant increase of cholesterol-7α-hydroxylase activity is found 16 h after administration of cholestyramine (Fig. 2).

During the following time enzyme activity does not remain on an enhanced level but oscillates in an increased rhythm with synchronous minima and maxima. Withdrawal of bile acids from the enterohepatic circulation causes an enhanced cholesterol-7α-hydroxylase activity oscillating on a higher level.

The activation of cholesterol catabolism leads to a depletion of cholesterol. So it is conceivable that HMG-CoA reductase is activated by cholestyramine, too (Table 1).

Determinations at two times of day illustrate the enhanced rhythm in this case. HMG-CoA reductase activity was determined according to the method of Huber (1973a).

Table 1. HMG-CoA reductase activity (rel) $\pm$ $s\bar{x}$ in untreated (controls) and in cholestyramine-treated rats

Treatment	9.00 a.m.	11.00 p.m.
Control	1.0 $\pm$ 0.1	2.5 $\pm$ 0.3
Cholestyramine	2.3 $\pm$ 0.2	3.2 $\pm$ 0.1

Food and water ad lib., 3 rats per point. Cholestyramine: 85 mg/100 g body weight via intragastric tube, administration every 12 h, first 24 h before experiment

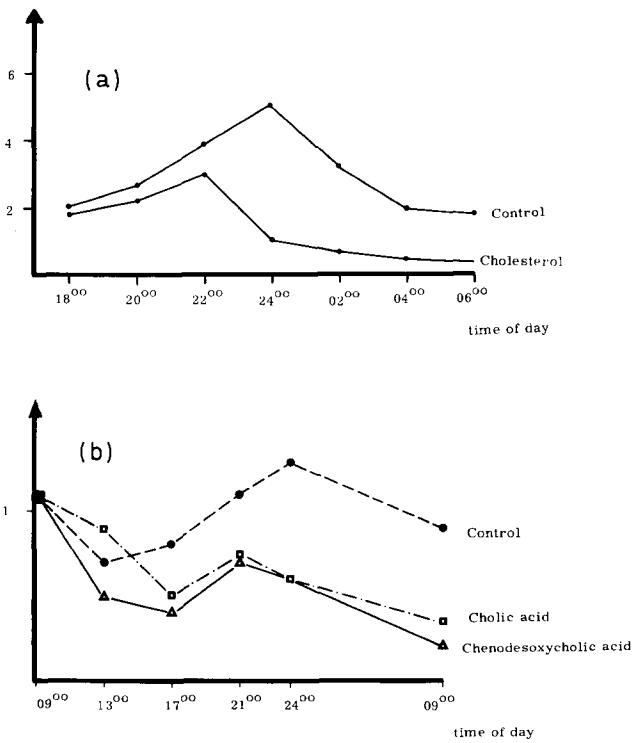

Fig. 3. **a** Course of HMG-CoA reductase activity (nmol/mg/h) in untreated rats (controls) and in rats fed a cholesterol diet (1%); food and water ad lib. Cholesterol diet was fed from the beginning of experiment (light 06.00–18.00; dark 18.00–06.00) (reproduced from Higgins, 1973). **b** Course of cholesterol-7α-hydroxylase activity (rel) in untreated (controls) and in cholic acid or chenodesoxycholic acid-treated rats; food and water ad lib.; 4 rats per point. Administration of cholic acid or chenodesoxycholic acid: 25 mg/100 g body weight in a single dose, via intragastric tube, 1 h before experiment (light 07.00–19.00; dark 19.00–07.00)

Figure 3 demonstrates the negative feedback effects of products on their synthesis. Feeding of cholic acid or chenodesoxycholic acid leads to an increase of bile acids in the enterohepatic circulation (Fig. 3b) (Mayer, 1974a, and unpublished). A significant inhibition of cholesterol-7α-hydroxylase activity is seen 9 h after feeding

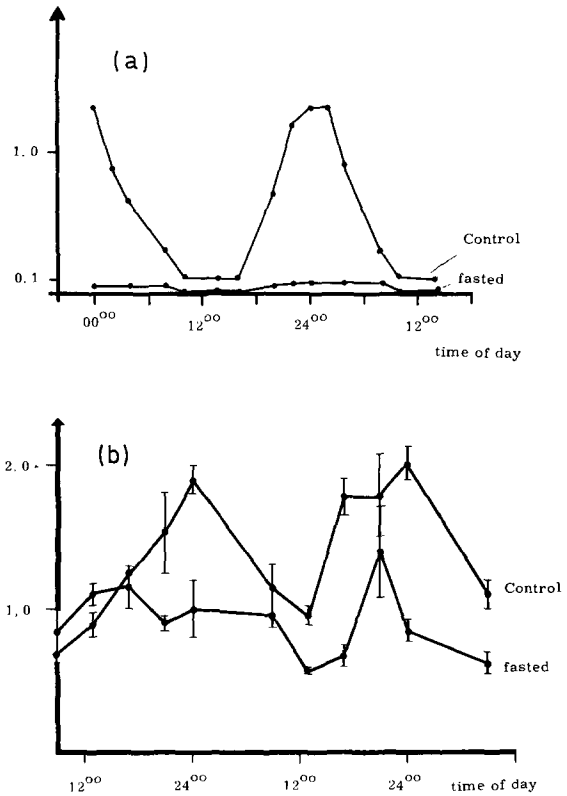

Fig. 4. **a** Course of HMG-CoA reductase activity [μ mol/g liver/h] ± $s\bar{x}$ in untreated (controls) and in fasted rats; 4 animals per point; at 24.00: 8 animals (reproduced from Hamprecht, 1969). **b** Cholesterol-7α-hydroxylase activity (rel). Starvation: 1 h before experiment (light 07.00–19.00; dark 19.00–07.00)

of bile acids compared to the rhythm of the controls (dotted line). After a certain time of adjusting the balance a distinct but damped rhythm has developed.

Feeding of cholesterol (Higgins, 1973) leads to an inhibition of HMG-CoA reductase (Fig. 3a). In this case, too, a damped rhythm reappears.

In the fasting state intestinal activity is lowered. This might lead to an increased reabsorption of bile acids. Combined with a lowered excretion it might lead to an increase of bile acid content in the enterohepatic circulation; thus the result of Figure 4 could be explained. In the fasting state cholesterol-7α-hydroxylase activity (Mayer, 1974a) is lowered significantly in the first dark period (Fig. 4b).

Whereas there is no maximum in the first day after starvation a rhythm develops during the second day. HMG-CoA reductase (Hamprecht 1969) is markedly lowered under the same conditions (Fig. 4a). When the measuring points are plotted on an expanded scale, as done by the author, a circadian rhythm with distinct maxima in the dark and minima in the light period appears.

The next example demonstrates once more that it is necessary to pay attention to the circadian rhythm when looking for any influence on cholesterol catabolism.

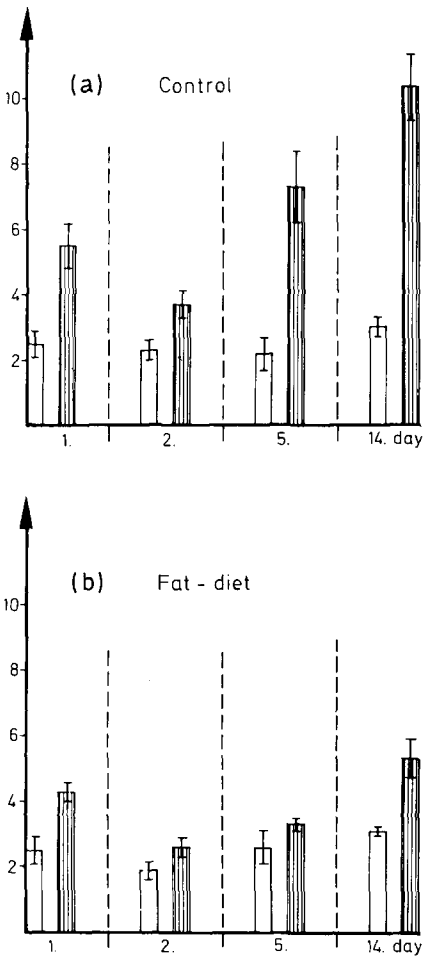

Fig. 5. Cholesterol-7α-hydroxylase activity (nmol 7α-hydroxycholesterol/50 mg liver/20 min) in rats on a standard diet [= controls; (**a**)] and on a 20% fat diet [saturated fatty acids predominantly (**b**)]. Determinations were done 1, 2, 5, and 14 days after change of diet. The white columns mean the 09.00 values, the cross columns the 23.00 values

Investigations done in our laboratory have shown that the circulation of bile acids can be influenced by fat diets of different fatty acid composition (Mayer, 1974b). Per circulation a distinct percentage of bile acids is excreted with the feces. Thus biosynthesis of bile acids signifying activity of cholesterol-7α-hydroxylase depends on the number of circulations per day.

Figure 5 demonstrates the course of cholesterol-7α-hydroxylase activity at two times of day in rats on a standard and on a fat diet. In Figure 5a the circadian rhythm of the controls is to be seen clearly at all days of the experiment. At the beginning of the experiment the animals were fed a 20% fat diet containing saturated fatty acids predominantly (Fig. 5b). Here the maximal values are markedly lowered whereas the 9.00 a.m. values remain nearly unchanged.

Table 2. Cholesterol-7α-hydroxylase activity (rel) $\pm$ $s\bar{x}$ according to time of day

Treatment	9.00 a.m.	11.00 p.m.
Control	1.0 $\pm$ 0.1	2.5 $\pm$ 0.2
Thyroidectomy	0.7 $\pm$ 0.1	1.9 $\pm$ 0.2
Thyroxin	0.9 $\pm$ 0.1	3.0 $\pm$ 0.3
Thyroidectomy thyroxin	1.3 $\pm$ 0.3	2.6 $\pm$ 0.3

Food and water ad lib., 4 animals per point. *In untreated rats* (controls); *In thyroidectomised rats:* radiothyroidectomy 6 weeks before experiment by 0.8 mCl $Na^{131}I$; *In thyroxin-treated rats:* 20 µg/100 g body weight, i.p. on days 15, 12, 9, 6, and 2 before experiment; *In thyroxin-treated thyroidectomised rats:* radiothyroidectomy 6 weeks before experiment by 0.8 mCi $Na^{131}I$; thyroxin µg/100 g body weight, i.p. at the 15, 12, 9, 6, and 2 days before experiment

This result indicates that a possible influence of any substance on cholesterol turnover might have failed in this case; enzyme activities are determined only once per day.

All experiments discussed so far demonstrate that bile acids are a factor in the regulation of cholesterol turnover – synthesis as well as catabolism – by modifying the rhythm of the rate-limiting enzymes. These are influenced synergistically.

Next we will discuss the influence of hormones on cholesterol turnover. How do they influence the rhythm of the two regulating enzymes?

The influence of thyroxin, insulin, and hypophysectomy on cholesterol-7α-hydroxylase and HMG-CoA reductase has been described.

The effect of thyroxin on 7α-hydroxylase activity was investigated in three series in the rat: in a state of thyroxin deficiency (after radiothyroidectomy), after thyroxin administration in the normal rat and after hormone substitution in the thyroidectomised animal. Determinations were carried out at 9.00 a.m. (low enzyme activity) and at 11.00 p.m. (high enzyme activity). The results are summarised in Table 2.

In a state of thyroxin deficiency a damped rhythmic course of activity is observed with nearly unchanged low values. The inhibition is caused by a decrease of amplitude only. Thyroxin treatment of the normal remains without influence on cholesterol-7α-hydroxylase activity. Hormone substitution in the thyroidectomised rat leads to normalised values.

After thyroidectomy HMG-CoA reductase activity is inhibited, too. After thyroxin substitution an activation was observed (Guder, 1968).

Insulin is the first hormone (investigated here) to exhibit different influences on biosynthesis and catabolism of cholesterol. In this case, our own results on cholesterol-7α-hydroxylase activity are compared to those of other authors on HMG-CoA reductase obtained in analogous experiments. The results concerning 7α-hydroxylase are listed in Table 3.

Four days insulin treatment (administration at 4.00 p.m. every day) leads to an inhibition of 7α-hydroxylase in the normal with a significant decrease in both dark and the light period values. Six hours after administration of insulin in the normal,

Table 3. Cholesterol-7α-hydroxylase activity (rel) $\pm$ $s\bar{x}$ according to time of day

Treatment	9.00 a.m.	11.00 p.m.
Control	1.0 $\pm$ 0.2	2.7 $\pm$ 0.2
Insulin	0.6 $\pm$ 0.1	2.3 $\pm$ 0.2
Diabetes	0.7 $\pm$ 0.1	4.1 $\pm$ 0.7
Diabetes insulin	0.9 $\pm$ 0.1	2.0 $\pm$ 0.3

Food and water ad lib., 3 animals per point. *In untreated rats* (controls); *In insulin treated rats:* 3 U/100 g body weight s.c., on days 4, 3, 2, and 1 before experiment at 16.00; *In diabetic rats:* 6.5 mg streptozotocin/100 g body weight, i.v. in a single dose 7 days before experiment; *In insulin-treated diabetic rats:* 3 U/100 g body weight, s.c. on days 4, 3, 2, and 1 before experiment at 16.00; 6.5 mg streptozotocin/100 g body weight i.v. in a single dose 7 days before experiment

HMG-CoA reductase remains uninfluenced (Laksmanan, 1973). The diabetic state (7 days after streptozotocin) leads to an activation of cholesterol-7α-hydroxylase with an enlarged amplitude. Under the same conditions HMG-CoA reductase is inhibited nearly completely (Huber, 1973b; Dugan, 1974). A rhythm does not become evident then. Four-days substitution of insulin in chronic diabetes leads to a significant inhibition of cholesterol-7α-hydroxylase. The results are similar to those of the insulin-treated normal. In analogous experiments HMG-CoA reductase shows normal values (Huber, 1973b; Dugan, 1974).

According to these results it can be said that insulin has a modifying effect on the rhythm. Since this hormone has opposite influences on the two regulating enzymes of cholesterol turnover, it could serve as an instrument of regulation.

Inasmuch as in all experiments so far the rhythm could not be eliminated, it was interesting to investigate the enzymes in hypophysectomised animals (Fig. 6).

A significant oscillation cannot be demonstrated (Gielen, 1970; Mayer, 1972b). This is in contrast to the marked rhythm of the controls (Fig. 1). Nor does HMG-CoA reductase show different activities during dark and light periods (Edwards, 1973).

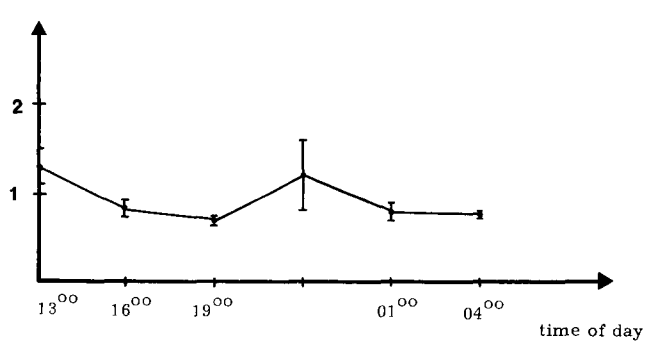

Fig. 6. Course of cholesterol-7α-hydroxylase activity $\pm$ $s\bar{x}$ 7 days after hypophysectomy; food and water (5% glucose) ad lib. (light 07.00–19.00; dark 19.00–07.00)

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