RHYTHMIC CHANGES OF HYDROXYMETHYLGLUTARYL COENZYME A REDUCTASE ACTIVITY IN LIVERS OF FED AND FASTED RATS

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1. Introduction

During studies on the cholesterol biosynthetic activity in liver slices of rats kept under different nutritional conditions, we found that the rate of acetate incorporation into cholesterol depends on the time of day when the rats were killed. The activity exhibits a diurnal rhythm with the highest values at midnight and the lowest at noon [1,2]. Using a new sensitive radiogaschromatographic assay procedure for the microsomal enzyme HMG-CoA reductase (β-hydroxy-β-methylglutaryl-CoA reductase, E.C. 1.1.1.34) [3–5] we could demonstrate similar rhythmic changes for this enzyme [3]. This was expected since in other studies HMG-CoA reductase was demonstrated to be the rate limiting enzyme of cholesterol biosynthesis from acetate [1,6,7]. Therefore the regulation of cholesterol synthesis is most effectively exerted at this step. As rats feed mainly at night, investigations were made whether the rhythm existed independently of food intake. Livers from fasted rats have extremely low activities of HMG-CoA reductase. The difficulty in detecting significant differences in these low levels did not arise, when the new test procedure was used. A recent publication [8] described the increase, during the time between 8.30 and 20.30 hr, in the incorporation of acetate into liver 3β-sterols of mice fed ad libitum. This increase agrees with the discovery in this laboratory of a 24 hr cycle of cholesterol synthetic activity in rat liver. These authors also confirm our findings of the time dependence of HMG-CoA reductase [3], when they show the activity of this enzyme at 16.30 hr to be 5.5 times higher than that at 8 hr.

In this communication it is shown that the activity of HMG-CoA reductase underlies similar diurnal rhythmicity in both livers of normally fed and of fasted rats.

2. Experimental

Male Sprague-Dawley rats of 90 to 150 g body weight (Fa. Wiga, Ottobrunn/Munich) were kept at 23°C in wire cages with free access to standardized rat chow Altromin R 15 (Fa. Altrogge, Lage/Lippe) in a room that was artificially illuminated from 7.00 to 19.00 hr. The supplied animals, which were adapted to their new environment for at least 6 days, showed normal weight increases. Fasted rats were without food for 24 hr before sacrifice. Groups of fasted and of fed rats to be used for measuring HMG-CoA reductase activity at each specific time were killed within 10–14 min of the given time and on the same day.

The preparation of (5-^{14}C)-HMG-CoA and of microsomes and the procedure for protein determination have been previously described [9]. The details of the radiogaschromatographic assay developed for determination of HMG-CoA reductase are given elsewhere [3–5].

3. Results and discussion

To ensure that HMG-CoA reductase activities in all groups of experimental animals could be accurately compared, we determined enzyme activities at 4 dif-

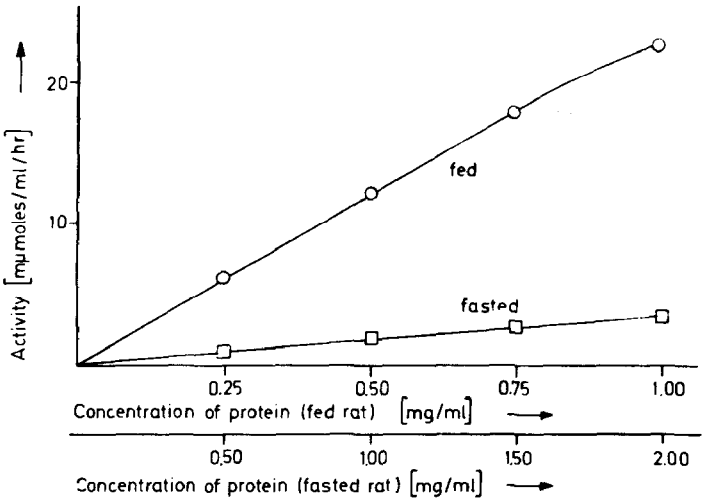

Fig. 1. Relationship between activity of HMG-CoA reductase and concentration of protein with fed and fasted rats. Each curve represents the results obtained with hepatic microsomes from one animal. The rats were sacrificed at 4 hr.

ferent concentrations of microsomal protein using one rat from each group, which normally consisted of 4 rats. The activities increased linearly with protein concentration in the incubation mixtures in nearly all cases. The only exceptions were fasted rats at 12, 14, 16 and 18 hr, where the specific activities were so low, that they could no longer be discerned from zero. In some cases, at the highest protein concentration used, slight deviation from linearity, i.e. proportionally lower values, was observed. With the other rats of each group, assays were run at only one protein concentration corresponding to the midpoints of the protein dependency curves which were obtained with microsomes of the trial rats of each particular group (fig. 1). As it is not possible to present enzyme activity/protein concentration curves for every group of rats, only two curves out of a total of 24 are given here (fig. 1).

From fig. 2a it may be seen that maximum specific activity of HMG-CoA reductase is reached in the middle of the dark period at 2.00 hr. The activity then declines to a broad minimum between 10.00 and 16.00 hr. In the evening specific activity again rises. The activity at 2.00 hr exceeds that at 14.00 by a factor of 13.2. As a basis for the calculations of specific activity in fig. 2a the protein content of the microsomal suspension was used. Objections may be raised that the specific activity of the enzyme could increase, while the total activity per unit weight of liver nevertheless remains rather constant. In fig. 2b however, specific activities expressed as mμmoles mevalonate formed per gram liver per hour show a diurnal rhythm very similar to that on the protein basis (fig. 2a). Furthermore, it is not possible to explain even part of this rhythmicity by a diurnal change of liver weight per 100 grams rat body weight, since the upper curve in fig. 3 shows maximal differences of normalized liver weights of only 25 per cent (values at 8.00 and at 20.00 hr) in comparison to over 1300 per cent change in specific activity (values at 2.00 and at 14.00 hr). The broken line at the bottom of fig. 2a marks the upper limit of specific activity of HMG-CoA reductase found in rats having been fasted. If this region is enlarged to the scale shown in fig. 4a, a plot of specific activities against the times of day reveals a diurnal rhythm also in the case of fasted rats which is very similar to that observed with animals fed ad libitum. The character of the rhythm appears unchanged, when again (as in fig. 2b) the activities are expressed on the basis of unit weight of liver (fig. 4b). The lower curve in fig. 3 not only confirms the well known fact that with fasted rats the liver weight per 100 grams of body weight is much lower than with fed animals [10], but it also demonstrates a greatly reduced rhythmicity of that variable.

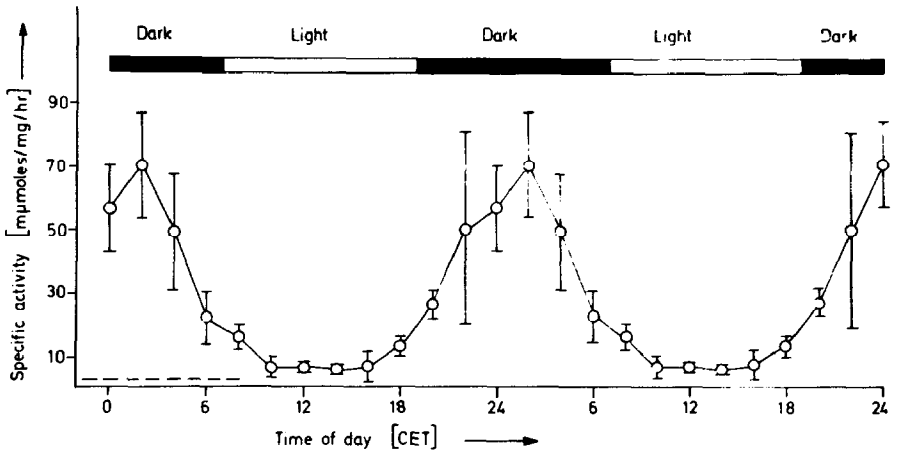

Fig. 2a. Activity per mg microsomal protein per hour.

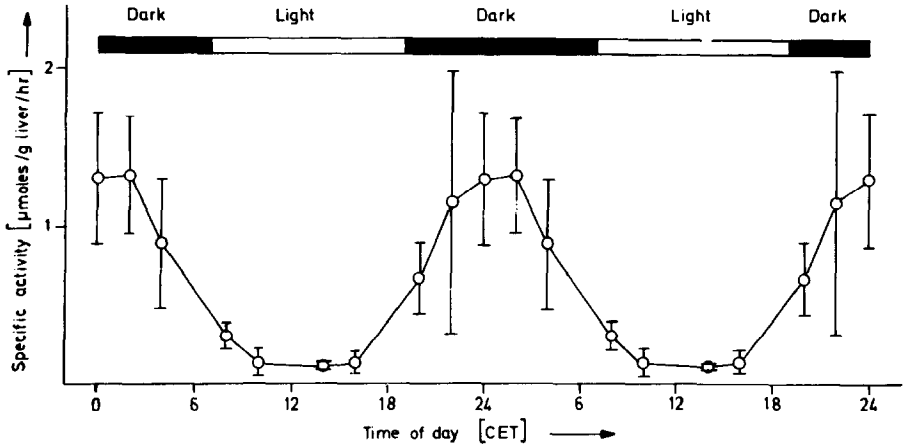

Fig. 2b. Activity per g liver wet weight per hour.

Diurnal rhythm of specific activity of hepatic HMG-CoA reductase from rats fed ad libitum. Each point is the mean of values from 4 rats except the point at 10.00 hr, which was obtained with 3 rats. At each point the standard deviation of the mean is indicated by the vertical line. The points have been plotted over a time period of 48 hr to convey a better impression of the rhythmic process.

For several years the regulation of hepatic cholesterol biosynthesis via *de novo* synthesis and degradation of HMG-CoA reductase has been considered [6,9]. This view prevailed when investigations to find a feedback-inhibitor of HMG-CoA reductase proved to be negative. In addition it could be shown that the depressing effect of bile acids on cholesterol biosynthesis *in vivo* is not due to feedback inhibition of HMG-CoA reductase [2–4]. This provided another hint to concentrate on the level of protein synthesis and degradation, when studying the regulation of cholesterol biosynthesis. Refeeding experiments on fasted rats on the other hand already had provided further evidence for this hypothesis, since the increase in the rate of acetate incorporation into cholesterol by liver slices was prevented when puromycin had

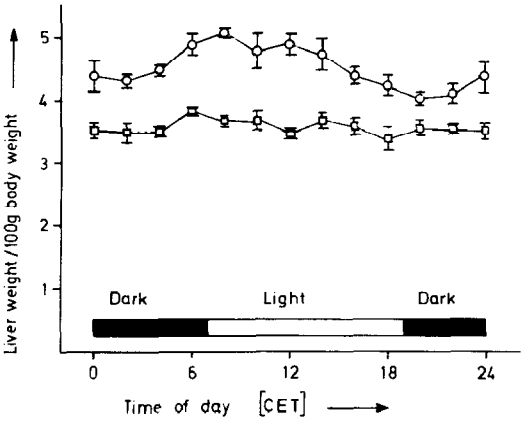

Fig. 3. Normalized liver wet weight of rats as a function of time of day. Upper curve: Rats fed, lower curve: rats starved 24 hr. The points indicate mean values from 4 rats, except those at 0.00 hr, were 8 animals were used. The vertical lines represent the standard deviations of the mean.

been injected during the refeeding period [3]. Back [1] then showed that the increase of cholesterol synthetic activity in rats at night was prevented by injection of cycloheximide. This antibiotic is known to inhibit protein biosynthesis on the translational level. Other studies on different aspects of control of cholesterol synthesis using actinomycin D and puromycin as agents to alter protein synthesis supplied further evidence for regulation on the level of protein synthesis [8,11–13]. In the context of all these findings it seems to us to be important that fasted rats also show a diurnal rhythm of HMG-CoA reductase activity. This would mean that the rhythm cannot be caused by the intake of food in the dark period. The rhythmic activity of cholesterol synthesis appears to be a permanent feature in all nutritional states, although its magnitude is greatly increased after feeding. Hormonal regulation of the rhythm seems to be a strong possibility when one considers evidence already available from the literature. Thyroid hormones [14] and especially norepinephrine [13] seem to be good candidates. The latter's importance in the daily rhythm of tyrosine transaminase has been convincingly established [15].

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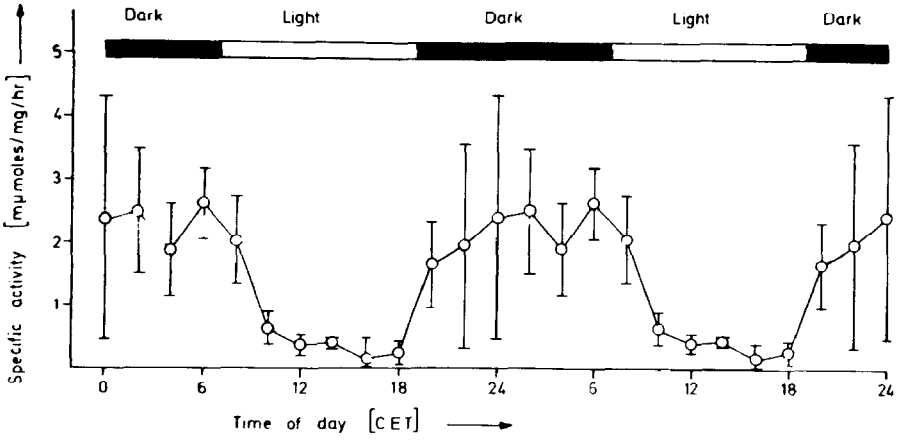

Fig. 4a. Activity per mg microsomal protein per hour.

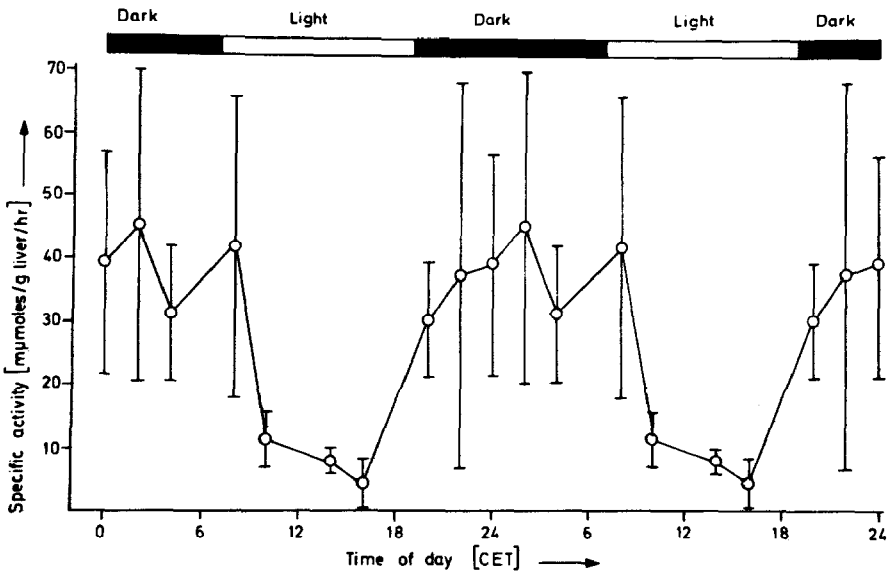

Fig. 4b. Activity per g liver wet weight per hour.
Diurnal rhythm of specific activity of hepatic HMG-CoA reductase from rats fasted 24 hr. Each point is the mean of values from 4 rats, except the point at 0.00 hr, where 8 rats were used. Other details as in fig. 2.

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