Measurement of hepatic sterol synthesis in the Mongolian gerbil in vivo using [^{3}H]water: diurnal variation and effect of type of dietary fat

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Abstract The hepatic synthesis of sterol was measured in the male Mongolian gerbil (*Meriones unguiculatus*) in vivo following the administration of [^{3}H]water by monitoring the incorporation of radioactivity into digitonin-precipitable sterol. A diurnal rhythm in cholesterol synthesis was exhibited under conditions of ad libitum feeding with alternating 12-hour periods of light (0200 to 1400 hr) and dark (1400 to 0200 hr). The zenith was reached between 1500 and 2100 hr and the nadir approximately 10–12 hours later between 0200 and 0400 hr, which provided a zenith/nadir ratio of 9.6 to 1.0. The in vivo rates of hepatic sterol synthesis and plasma cholesterol levels were measured in gerbils fed semi-purified diets containing either 19.5% beef tallow + 0.5% safflower, 20% lard, or 20% safflower oil and widely differing ratios of polyunsaturated: saturated fatty acids. All diets were equalized to contain 0.01% cholesterol and 0.05% plant sterol. After 3 days on the experimental diets, the mean rates of cholesterol synthesis (nmol/g liver per hr) were 41.5, 26.6, and 13.8 for animals fed the diets containing beef tallow, lard, and safflower oil, respectively. After 7 and 14 days, synthetic rates were lowest in the gerbils fed safflower oil as were also the plasma cholesterol levels. These results indicate that the type of dietary lipid can significantly influence the in vivo rate of sterol biosynthesis in gerbil liver. This response may contribute, at least in part, to the observed differences in plasma cholesterol levels.—**Mercer, N. J. H., and B. J. Holub.** Measurement of hepatic sterol synthesis in the Mongolian gerbil in vivo using [^{3}H]water: diurnal va iation and effect of type of dietary fat. *J. Lipid Res.* 1981. **22:** 792–799.

Supplementary key words liver cholesterol synthesis · dietary lipid · diurnal variation in cholesterol synthesis

A variety of dietary factors appears to have an effect on the regulation of plasma cholesterol levels. Of these, the fatty acid pattern of the dietary lipid appears to have a significant influence (1, 2). Over the past decade numerous studies have established the fact that the feeding of unsaturated fats to humans has a hypocholesterolemic effect, while saturated fats have a hypercholesterolemic action (1, 2). Recently, Mercer and Holub (3) have demonstrated the same type of plasma cholesterol response in gerbils fed diets with differing ratios of polyunsaturated:saturated fatty acids.

The mechanism(s) whereby unsaturated fats reduce plasma cholesterol levels remains an enigma despite extensive research conducted in this area. Among others, Nestel et al. (4) have suggested that unsaturated fats cause an increase in fecal excretion of neutral and acidic sterols, thereby producing a secondary decrease in plasma cholesterol. Grundy and Ahrens (5) have calculated that a redistribution of cholesterol within the body occurs when polyunsaturated fatty acids (PUFA) are fed, thereby causing a shift of cholesterol from the plasma into tissue pools. In a recent study, Shepherd et al. (6) observed that a polyunsaturated fat diet did not cause a consistent change in fecal neutral or acidic steroid excretion in human subjects and concluded that the hypocholesterolemic action of polyunsaturated fats may be effected by multiple mechanisms. Since the liver is the major site of endogenous cholesterol synthesis (7), it is possible that the type of dietary lipid may influence the rate of cholesterol biogenesis. In this regard, Ide, Okamatsu, and Sugano (8) have reported that the activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase [EC 1.1.1.34] in rat liver was higher in animals fed the saturated fat, tristearin, as compared to those given the polyunsaturated fat, safflower oil. However, the plasma cholesterol levels were found to be significantly higher in the safflower oil-fed rats. Also, no attempt was made to equalize other dietary fats for the presence of endogenous plant sterol in the safflower oil. There is evidence to indicate that dietary plant sterol

Abbreviations: DPS, digitonin-precipitable sterol; PUFA, polyunsaturated fatty acids; P/S, ratio of polyunsaturated to saturated fatty acids in the dietary fat; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; GLC, gas–liquid chromatography.

may influence HMG-CoA reductase activity in rat liver microsomes (9, 10). Raicht et al. (9) reported that the inclusion of 0.8% β-sitosterol in rat diets led to a two-fold increase in HMG-CoA reductase activity. A significantly greater activity of liver HMG-CoA reductase activity was observed by Bochenek and Rodgers (11) when rats were fed a saturated fat diet plus 1% cholesterol compared to rats fed an unsaturated fat diet plus 1% cholesterol, although plasma cholesterol levels were not reported.

Most measurements of rates of cholesterol synthesis have been made under in vitro conditions using ^{14}C-labeled substrates with whole cell preparations such as tissue slices, isolated cells, tissue homogenates, or isolated microsomes. Each of these approaches has advantages and disadvantages that, according to Andersen and Dietschy (12) and Jeske and Dietschy (13), at best allow for the estimation of relative rates of cholesterol synthesis in vitro but do not allow for valid in vivo determination of sterol synthesis. Some investigators (13–15) have observed that in vivo sterol synthetic rates are considerably different from rates observed when in vitro techniques are employed.

There is now strong support for the use of [^3H]-water to best assess the rates of cholesterol synthesis in vivo (12–16). Using this approach, Triscari, Hamilton, and Sullivan (17) reported that prolonged feeding of a completely saturated fat would increase hepatic cholesterol synthesis in the rat. A decrease in plasma cholesterol levels was observed in the rats fed diets containing 20% by weight of saturated fat relative to rats fed diets containing 20% by weight of corn oil. This reported response of plasma cholesterol concentrations to manipulation of dietary lipids is characteristic for the rat and different from that observed with humans (1, 2, 4) and the Mongolian gerbil (3).

The human-like response of plasma cholesterol levels in the Mongolian gerbil (*Meriones unguiculatus*) to changes in dietary fatty acid patterns has recently been documented by Mercer and Holub (3). This particular animal model would appear to lend itself favorably to studies on the possible effects of dietary lipid on cholesterol metabolism. Thus, the present experiments were initiated to determine if the types of dietary fat may influence the in vivo rates of hepatic cholesterol synthesis in the gerbil using [^3H]water. It was also of considerable interest to determine if a diurnal cycle in the rates of cholesterol biosynthesis is present in the gerbil in vivo as reported to exist in rat liver based on in vitro experiments (18). All diets were formulated to contain 20% by weight of fat with equal amounts of both cholesterol and plant sterol.

MATERIALS AND METHODS

Animals

Male Mongolian gerbils (*Meriones unguiculatus*) were purchased from High Oak Ranch, Goodwood, Ontario in the weight range of 37–45 g. Animals were housed, two per wire-bottomed, stainless steel cage, in a room that was artificially illuminated from 0200 to 1400 hr. All animals were allowed free access to food and water, and body weight gain and food intake were recorded every 3–4 days. In the case of studies on the diurnal variation in the rate of hepatic cholesterogenesis, gerbils were fed Purina rat chow before being transferred to a semi-purified diet containing lard (see below). For studying the effect of the type of dietary fat on hepatic cholesterol synthesis, animals were adapted to their environment for 7 days before experiments were begun, during which time they were fed the basal diet containing lard (see below). Most studies were carried out using animals killed at the mid-dark phase of the light cycle (1700 to 1900 hr), but in specific experiments such as the diurnal rhythm study, animals were also killed throughout the light and dark cycles.

Diets

Animals were fed one of the following experimental diets. All formulated diets contained 20% by weight fat which was either beef tallow, lard, or safflower oil. The animals fed the saturated fat (beef tallow) were given 19.5% beef tallow plus 0.5% safflower oil mixture to ensure adequate linoleic acid in the diet. Gas–liquid chromatographic (GLC) analysis of the fatty acid pattern and the cholesterol and/or plant sterol content of the fats and oils used in the diets was performed as previously described (3). All experimental diets were formulated by appropriate supplementation to contain equal amounts of cholesterol (0.01%) and plant sterol (0.05%). The diet composition and fatty acid patterns of the fats are reported in **Tables 1** and **2.**

Determination of plasma cholesterol

Blood was obtained from gerbils using the orbital sinus bleeding technique. Plasma samples (10 μl) were analyzed by the microcolorimetric method of Bhandaru et al. (19). For a number of samples, GLC analyses of plasma lipid profiles were carried out to confirm the accuracy of the microcolorimetric method (3). The two methods gave total plasma cholesterol levels which were within 1% of each other (n = 11).

TABLE 1. Composition of experimental diets[a]

Ingredients	Dietary Lipid		
	Beef Tallow	Lard	Safflower Oil
		g/kg	
Casein	160	160	160
Carbohydrate			
Sucrose	181.8	181.8	181.8
Corn starch	364	364	364
Fat[b]			
Lard		200	
Safflower oil	5		200
Beef tallow	195		

[a] All diets contained, as g/kg, Alfa Floc, 40.2; salt mix, Williams Briggs Modified, 40; vitamin mix, 10; choline chloride, 3; inositol, 1; chromium acetate, 0.0023; sodium selenite, 0.00022.

[b] All diets were equalized to contain 114 mg cholesterol/kg diet and 480 mg plant sterol/kg diet.

Preliminary studies

Preliminary studies were conducted to determine the length of in vivo exposure to [^{3}H]water required to ensure approximately linear rates of tritium incorporated into DPS. In these experiments, gerbils fed the three experimental diets were studied. The time study (**Fig. 1**) revealed a steady increase in tritium incorporation into hepatic sterol with increasing time (up to 2 hr) following the injection of [^{3}H]-water, whereas the specific activity of liver water remained essentially constant over this same interval. Based on these experiments, an exposure time of 2 hr was routinely used in all other experiments. It has also been concluded from experimentation in meal-fed rats that a 2-hr period following the intraperitoneal injection of [^{3}H]water is valid for calculating cholesterogenesis in vivo (16).

TABLE 2. Fatty acid composition of dietary fats[a]

Fatty Acid	Beef Tallow	Lard	Safflower Oil
14:0	3.8	2.8	trace
16:0	29.5	25.1	9.2
16:1	3.3	3.1	trace
18:0	22.9	14.6	2.2
18:1	34.2	39.8	11.2
18:2	6.3	13.0	77.4
20:0		1.1	
Total saturated fatty acids	56.2	43.6	11.4
P/S ratio[b]	0.11	0.30	6.79

[a] Values are given as mole % of the total. Fatty acids representing less than 0.5% of the total in any dietary lipid have been omitted from the table.

[b] Calculated as total % of polyunsaturated fatty acids divided by total % of saturated fatty acids.

In vivo incorporation of [^{3}H]water into digitonin-precipitable sterols

Gerbils were injected intraperitoneally with 0.9% saline containing [^{3}H]water (4 mCi/100 g body wt) (New England Nuclear, Boston, MA). After an initial experiment to determine suitable times for measuring the incorporation of [^{3}H]water into digitonin-precipitable sterols (DPS), animals were killed by cervical dislocation 2 hr after injection and livers were quickly removed, blotted dry, and weighed.

Livers were homogenized and extracted in 20 volumes of chloroform–methanol 2:1 using an Ultra-Turrax 182E Tissumizer (Tekmar Co., Cincinnati, OH). After homogenization, 0.2 volumes of water was added and the upper and lower phases were allowed to separate. The total upper phase was removed and a suitable aliquot of this aqueous phase was counted in Aquasol (New England Nuclear, Boston, MA) to determine the specific activity of the liver water and to ensure that equilibration of the injected [^{3}H]water with liver water pools had occurred. The total chloroform phase was evaporated, dissolved in 5 ml of ethanol, and saponified with 10 ml of 5 N

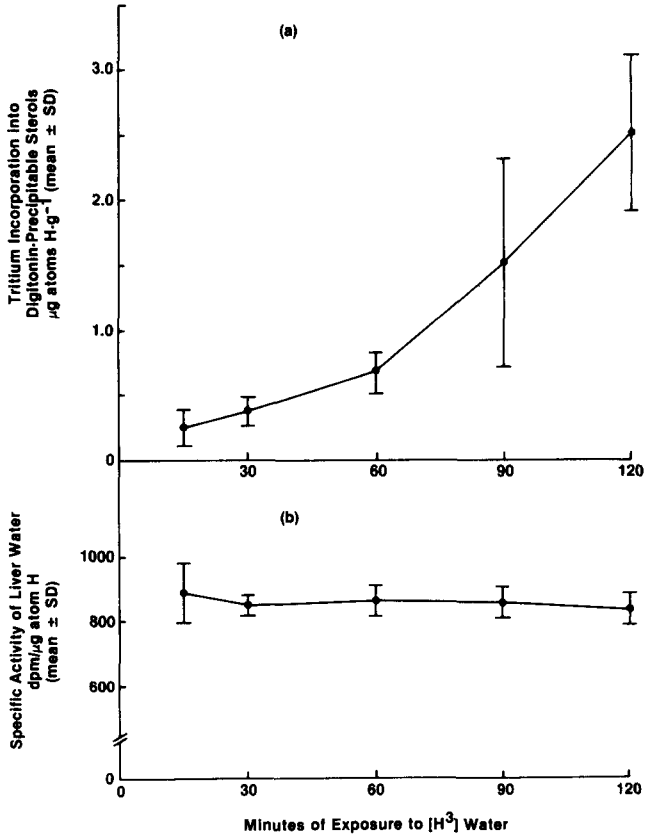

Fig. 1. Incorporation of tritium into hepatic sterol (a) and specific activity of liver water (b) following the intraperitoneal injection of [^{3}H]water.

NaOH at 80°C for 3 hr (16, 20). The non-saponifiable fraction was extracted three times with 15 ml of petroleum ether (bp 38.0–45.9°C) and the pooled extracts were washed free of alkali with two 10-ml water extractions, and finally washed with 15 ml of 50% ethanol to remove any traces of [^{3}H]water. The washed ether extract was evaporated to dryness under nitrogen. The residue was dissolved in 10 ml of acetone–ethanol 1:1, acidified with 10% acetic acid, and the sterol fraction was precipitated with 5 ml of 0.5% digitonin (Sigma Chemical Company, St. Louis, MO) in 50% ethanol (21). After standing overnight at 5°C, the DPS fraction was washed two times with 15 ml of acetone–ether 1:2, followed by 10 ml of ether. The sample was centrifuged, and the supernatant was removed by Pasteur pipette. The pellet was dissolved in 1 ml of methanol and counted in Aquasol in a Delta 300 liquid scintillation counter (Searle Analytic Inc., Des Plaines, IL). Counts were corrected to 100% efficiency using the external standard-channels ratio method for quench correction.

Calculations

The water content of gerbil liver was determined by finding the mean dry weight of the liver (23.1%). The value of 76.9% liver water was used to calculate the specific activity of liver water as disintegrations per minute per microgram atom of H from water. Background blanks consisting of 1–2 g liver samples spiked with appropriate amounts of [^{3}H]water, corresponding to the radioactivity found in liver samples after in vivo injection, were carried through all DPS isolation procedures. These blank values were subtracted from the total radioactivity in DPS for all samples obtained from experimental animals and represented 1–10% of the total incorporation. The radioactivity in the DPS was divided by the weight of the liver (g), and by the duration of exposure to [^{3}H]water in vivo (2 hr), to give the total radioactivity in DPS as dpm of tritium incorporated into DPS per gram of liver per hour. This value was then divided by the specific activity of the liver water to express tritium incorporation into DPS as μg atom/g per hr. The cholesterol synthetic rate was then calculated by dividing the μg atom tritium incorporation into DPS/g per hr by 24.0. The value of 24.0 tritium atoms incorporated during the complete biosynthesis of cholesterol from acetyl-CoA has been derived by Andersen and Dietschy (12) using [1-^{14}C]-octanoate and [^{3}H]water. The calculated cholesterol synthetic rate was then expressed as nmol/g per hr.

TABLE 3. Effect of dietary lipid on food intake and weight gain of gerbils[a]

Diet	Cumulative Food Intake/Animal	Cumulative Weight Gain/Animal	Rates (Gain:Feed)
Beef tallow	67.8[b] ± 2.2	15.3[b] ± 0.6	0.23[b] ± 0.01
Lard	58.5[c] ± 1.8	14.3[b] ± 0.8	0.24[b] ± 0.01
Safflower oil	60.2[c] ± 1.7	12.7[b] ± 1.4	0.21[b] ± 0.04

[a] All data represent mean values ± SE for the first 7 days after transfer to the three experimental diets.
[b,c] Values in each column not sharing a common letter are significantly different at $P < 0.05$.

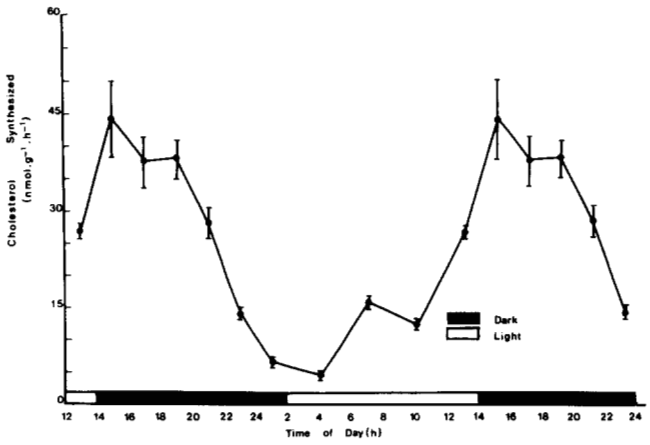

Fig. 2. Diurnal variation in the rate of hepatic cholesterol synthesis as measured by [^{3}H]water incorporation into [^{3}H]sterol. Mongolian gerbils were adapted to controlled light cycling and fed ad libitum a semipurified diet containing 20% by weight lard (plus plant sterol). Each point represents the mean ± SE for three animals.

RESULTS

Animals fed the three different semi-purified diets (Tables 1 and 2) showed similar cumulative food intakes and weight gains (**Table 3**). The gain:feed ratios were not significantly different.

A thorough study was undertaken to determine if the gerbil exhibited a diurnal rhythm in sterol biosynthesis as measured in vivo using [^{3}H]water. The gerbils used in the diurnal study had been fed the 20% lard diet (see Materials and Methods) ad libitum for 10 days prior to the study. Gerbils housed under a controlled lighting schedule with a 1400 to 0200-hr dark period exhibited a diurnal rhythm in the rate of hepatic [^{3}H]sterol synthesis as shown in **Fig. 2.** The nadir of cholesterol synthesis occurred between 0200 and 0400 hr and the zenith occurred between 1500 to 2100 hr, approximately 10–12 hr after the nadir. This timing of minimum and maximum [^{3}H]water incorporation into DPS corresponds well with the timing of the hepatic microsomal HMG-

TABLE 4. Effect of dietary lipid on the in vivo incorporation of [^{3}H]water into hepatic cholesterol in gerbils fed ad libitum[a]

Diet	Days on Diet	Rate of Tritium Incorporation into Cholesterol	Rate of Cholesterol Synthesis	Total Plasma Cholesterol
		μg atoms·g^{-1}·hr^{-1}	*nmol·g^{-1}·hr^{-1}*	*mg/100 ml*
—[b]	0	0.646 ± 0.008	26.96 ± 0.34	111.8 ± 5.9
Beef tallow	3	0.995^c ± 0.080	41.48^c ± 3.37	92.3^{cd} ± 5.8
Lard	3	0.638^d ± 0.036	26.59^d ± 1.50	101.8^c ± 5.5
Safflower oil	3	0.430^e ± 0.036	13.78^e ± 1.52	79.3^d ± 2.3
Beef tallow	7	0.769^c ± 0.085	32.07^c ± 3.58	123.3^c ± 2.1
Lard	7	0.627^c ± 0.037	26.14^c ± 1.58	103.8^d ± 3.2
Safflower oil	7	0.364^d ± 0.048	17.91^d ± 2.00	67.0^e ± 2.1
Beef tallow	14	0.392^c ± 0.021	16.33^c ± 0.91	134.3^c ± 3.4
Lard	14	0.522^d ± 0.046	21.75^d ± 1.95	97.6^d ± 3.6
Safflower oil	14	0.331^c ± 0.031	13.80^c ± 1.29	71.2^e ± 2.3

[a] All data represent mean values ± SE for six animals except those for total plasma cholesterol at days 7 and 14 where n = 10.

[b] All animals had been maintained on the lard diet for 7 days prior to transferring onto the three experimental diets.

[c,d,e] Values in each column at each time not sharing a common letter are significantly different at $P < 0.05$.

CoA reductase as reported by Edwards and Gould (22) and others (23, 24).

The effect of varying the fatty acid pattern of the dietary lipid on the in vivo incorporation of [^{3}H]-water into hepatic cholesterol is shown in **Table 4.** Total plasma cholesterol levels for the three dietary groups are also reported in Table 4. All gerbils were fed the 20% by weight lard diet for a 7-day preliminary period, at which time samples were taken and recorded as zero time. After this initial period, all animals were assigned randomly to one of the three dietary treatment groups and samples were taken after 3, 7, and 14 days on the dietary regimens. Gerbils fed the diet containing 20% lard exhibited biosynthetic rates that were quite similar (within 15% of each other) throughout the experiment. Furthermore, plasma cholesterol levels remained nearly constant over this period for the lard-fed animals. After 3 and 7 days on the experimental diets, the animals fed the beef tallow (saturated fat, P/S ratio 0.11) had the highest cholesterol synthetic rates. The latter group also had the most elevated plasma cholesterol levels on days 7 and 14. Dramatic differences in the rates of sterol synthesis across the groups were observed. After 3 days on the different diets, the beef tallow and lard groups had in vivo rates of cholesterogenesis that were greater by 200% and 93%, respectively, than those for animals consuming safflower oil. Gerbils consuming the diet with a high level of unsaturated fatty acids (safflower oil, P/S ratio 6.79) showed a significantly lower rate of cholesterol synthesis on days 3 and 7 than did either the lard- or beef tallow-fed animals. Plasma cholesterol levels were lowest in the animals consuming safflower oil at days 3, 7, and 14. By day 14, synthetic rates in the beef tallow animals had reduced to levels that approached those exhibited by gerbils consuming safflower oil.

DISCUSSION

Liver is generally believed to be a major source of circulating plasma cholesterol in normal animals and man. The regulation of hepatic sterol synthesis has been the subject of intense investigation over the past 20 years. Since a number of steps in the biosynthesis of cholesterol involve the incorporation of hydrogen atoms from water, the use of [^{3}H]water was chosen as the marked precursor to be followed when studying the in vivo rate of cholesterol synthesis. Tritiated water should be a better precursor than the usual ^{14}C-labeled substrates because it equilibrates rapidly with the labile hydrogens in the intermediates of cholesterol biosynthesis (12). Preliminary studies with the gerbil have demonstrated that, from 15 min up to 180 min, the specific activity of liver water remained nearly constant and in fact did not vary with the three experimental diets studied. This method, therefore, appears to be free of the uncertainty regarding variable precursor pool size and changing specific activity that are associated with the use of [^{14}C]acetate in the measurements of cholesterol synthesis in vivo (12, 15).

Edwards, Muroya, and Gould (25) demonstrated

an in vivo circadian rhythm in the rate of hepatic cholesterol synthesis in the rat by monitoring the conversion of [^{14}C]acetate to [^{14}C]cholesterol. Fears and Morgan (14), however, were unable to detect any postprandial influence on cholesterogenesis or any difference between the midday versus midnight incorporations of [^{3}H]water into the DPS of rat liver in vivo. This inability to detect a postprandial response to cholesterogenesis is contrary to reported increases in lipogenesis and cholesterogenesis in meal-fed rats versus rats fed ad libitum (26). The Mongolian gerbils used in the present study were fed ad libitum to ensure that the hepatic rates of cholesterogenesis determined were not stimulated by meal feeding. Andersen and Dietschy (27) reported that diurnal light cycling caused significant changes in the rate of in vitro cholesterogenesis in rats when [^{14}C]acetate was used to determine cholesterol synthesis in liver slices. This same study (27) also reported a mid-dark [^{3}H]water incorporation into DPS with tissue slices, but did not record data for a mid-light incorporation rate for comparison. Therefore, it seemed pertinent to study systematically the possible influence of the 12-hour light-dark cycles on the in vivo incorporation of [^{3}H]water into DPS in the gerbil. The peak of cholesterol synthesis in the male gerbil was found to occur during the dark period, approximately 10–12 hr after the nadir during the light period (Fig. 1). The timing of the cycling is nearly identical to that observed by Edwards and Gould (22) and others (23, 24, 27–29) who measured hepatic HMG-CoA reductase activity in rats fed Purina chow ad libitum. The amplitude of the rhythm, as defined by the ratio of the maximum to the minimum rates was approximately 10, and agrees with the approximately 12-fold lower activity of HMG-CoA reductase reported in rats fed ad libitum when the nadir was compared with the zenith (28). Ide et al. (8) have reported a mid-dark maximum rate of HMG-CoA reductase activity approximately six times the mid-light minimum rate in rats receiving 20% corn oil diets. This observation correlates well with the in vivo data for [^{3}H]water incorporation into DPS found herein when gerbils were fed a diet containing 20% lard. This study demonstrates that a diurnal rhythm does exist in the in vivo rate of hepatic cholesterogenesis in Mongolian gerbils fed ad libitum. It is of interest to note that the synthetic rate in liver, as reported by Jeske and Dietschy (13) for their mid-dark rats fed a rat chow diet, is approximately double that for mid-dark gerbils consuming a semi-purified diet containing lard (Fig. 1).

The major purpose of the present investigation was to determine the effect of different types of dietary lipid on rates of hepatic cholesterol synthesis in the gerbil in vivo. In this study, all diets were formulated to contain a constant level of cholesterol and plant sterol to eliminate the variable of changing levels of these sterols when different dietary fats and oils were substituted in the isocaloric experimental diets. The presence of exogenous cholesterol can have an effect not only on plasma cholesterol but it also causes marked suppression of hepatic cholesterol synthesis (7). In this study, the endogenous level of cholesterol present in the lard determined the maximum levels of exogenous cholesterol available in all diets. In this case, cholesterol was present in all diets at the level of 0.01%. This low level of cholesterol should not significantly affect the hepatic cholesterol synthetic rate, as Reiser et al. (10) was unable to demonstrate any change in HMG-CoA reductase activity with the addition of 0.05% cholesterol to a semipurified diet fed to rats. Reiser et al. (10) did show that the addition of 0.04% β-sitosterol increased the HMG-CoA reductase activity, although the increase did not reach statistical significance. It is possible that the 0.05% endogenous level of plant sterol present in the safflower oil that determined the level of plant sterol added to all diets could have had a slight effect on the rate of cholesterogenesis, but in this experiment the effect should be equal in all groups due to the equalization of diets to a constant plant sterol and cholesterol level. Measurements of sterol synthesis were conducted during the mid-dark phase of the light cycle (1700 to 1900 hr) corresponding to the period of maximal daily cholesterol synthesis established in the gerbil (Fig. 1).

The response at day 3 to the feeding of 20% safflower oil was a significant depression in the in vivo rate of hepatic cholesterol synthesis in the gerbil whereas the highly saturated fat (beef tallow) produced a significant increase in the synthetic rate when compared to the lard controls (Table 3). Triscari et al. (17) have reported that prolonged feeding of a completely saturated fat in the diet of rats will increase hepatic rates of cholesterogenesis from [^{3}H]-water, although 20% corn oil had no effect. These latter authors proposed that the effects on cholesterogenesis may have depended on the bioavailability of fatty acids supplied by the diet, which resulted in much lower growth rates in rats consuming the 20% hydrogenated soybean oil diet (98% saturated). It is highly unlikely that differences in fat absorption with the various diets influenced the results on sterol synthesis found in our investigations (Table 3), since the gerbils on these regimens (ranging from 11–56% saturated fatty acids) exhibited no difference in gain:feed ratios (Table 3). No differences in these

latter three parameters have been obtained in rats consuming their dietary fat as beef tallow or corn oil (30). The presence of 0.5 mM albumin-bound fatty acids ranging from 8:0 to 18:2 was reported to have no effect on [^{3}H]water-measured cholesterogenesis in isolated rat hepatocytes (31). The metabolic and biochemical changes related to cholesterol metabolism that result from dietary manipulation would be expected to manifest themselves at times before the differences in plasma cholesterol become readily apparent. In this regard, the following order was observed for relative cholesterol synthetic rates at both days 3 and 7: beef tallow > lard > safflower oil. The latter differences are in agreement with the relative differences in plasma cholesterol levels observed on days 7 and 14 but not as early as day 3 (Table 3). The fact that the rates of hepatic sterol synthesis across the dietary groups approach each other by day 14 suggests that plasma cholesterol levels might be expected to approach each other at later times. In fact, this expectation has been realized (3). Thus, our present results suggest that the effect of different types of dietary lipid on hepatic cholesterol synthesis in vivo may contribute, at least in part, to the observed differences in plasma cholesterol levels in the gerbil. Ide et al. (8) have reported that feeding tristearin to rats produced the highest HMG-CoA reductase activity of the C-18 fatty acids tested. A stimulatory effect of saturated dietary fats on hepatic HMG-CoA reductase may partly explain the increased rate of tritium incorporation into liver sterol observed in the gerbils fed beef tallow (Table 3). This concept remains to be investigated further, as do the many other potential mechanisms by which the type of dietary fat could influence plasma cholesterol levels in the gerbil.

This work was supported by a grant from the Ontario Heart Foundation.

Manuscript received 11 September 1980 and in revised form 2 February 1981.

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