Ability of Six Different Lipoprotein Fractions to Regulate the Rate of Hepatic Cholesterogenesis in Vivo*

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Two in vivo assay procedures were used to study the inhibitory activity of cholesterol carried in three intestinal lymph and three serum lipoprotein fractions on the rate of cholesterol synthesis in the liver. In the first preparation, different lipoproteins were injected intravenously as a bolus into rats at the mid-light phase of the diurnal light cycle, following which they were killed 12 hours later at the mid-dark phase of the cycle. Using this assay, three intestinal lymph lipoprotein fractions of varying S, values all produced a similar degree of inhibition which averaged approximately 11% per mg of cholesterol injected. The serum lipoprotein fractions caused only about one-third this amount of inhibition. Detailed analysis of events occurring within the liver during this 12-hour assay period revealed that there were marked differences in the rate of net cholesterol uptake into the liver and in the rate of net removal of cholesterol esters from the liver following injection of each of these different lipoprotein fractions. The amount of inhibition of sterol synthesis produced by any fraction was proportional to the product of the incremental increase in hepatic cholesterol ester content and the time over which this increase in esters occurred. In the second type of assay where the lipoprotein fractions were administered to the animals as a continuous intravenous infusion over 24 hours the largest increase in hepatic cholesterol ester content and the greatest inhibition of cholesterol synthesis was found with intestinal lipoproteins having S, values >8000. Intestinal lipoprotein fractions with lower S, values and all serum lipoprotein fractions were significantly less effective in bringing about an increase in hepatic cholesterol ester content and in producing inhibition of cholesterol synthesis by the liver. These studies emphasize the primary role of cholesterol carried in lipoproteins of intestinal origin in regulating hepatic sterol synthesis. The inhibitory activity of these fractions appears to correlate with the ability of these lipoproteins to bring about a maximal increase in hepatic cholesterol ester content which, in turn, appears to relate to the capacity of these fractions to transfer cholesterol rapidly into the hepatocyte while, at the same time, slowing the rate of cholesterol mobilization from the liver.

While the overall rate of cholesterogenesis is known to be regulated by the level of δ-hydroxy-δ-methylglutaryl-CoA reductase activity, there is little definitive information on the identity of the effector (or effectors) that communicate a given physiological variable such as stress or diurnal rhythms in lighting to the sites regulating the activity of this rate-limiting enzyme (1). In the case of cholesterol feeding, however, there is indirect evidence suggesting that cholesterol esters (or some closely related compound) in the hepatocyte may play such a regulatory role in adjusting the level of de novo cholesterol synthesis in the liver to the amount of exogenous sterol absorbed from the diet (2-4). The liver, however, is perfused with cholesterol carried in a variety of different types of lipoproteins, the levels of which may vary independently of one another under both normal and abnormal physiological circumstances. Thus, a better understanding of this important aspect of the regulation of sterol balance in the intact animal and in man critically depends upon elucidation of the role of each lipoprotein class in the regulation of hepatic cholesterol synthesis.

That there might be important differences in this regard between lipoproteins of different classes is suggested by the early observation of Sakakida et al. that serum from cholesterol-fed, but not from stilbesterol-injected, chickens inhibited cholesterol synthesis in the liver of the mouse (5). In the intervening 12 years little other relevant data have been published. The major problem in obtaining such information involves selection of an appropriate system in which to assay the inhibitory activity of the various lipoproteins. A minimum of several hours is required to produce inhibition of cholesterol synthesis so that attempts to use various liver preparations in vitro have been uniformly unsuccessful (6). Tissue culture
preparations of liver cells are usually derived from either fetal liver or from hepatomas and it is uncertain to what extent the response of such cells to inhibition by lipoproteins accurately reflects the process taking place in the normal cell. Furthermore, it probably is of critical importance that the anatomical relationships between the sinusoidal space, the space of Disse, and the sinusoidal membrane of the hepatocyte be maintained in any assay procedure since, in other tissues, it has now been shown that the rate of cellular uptake of lipid is determined critically by both the membrane and unstirred layer resistances (7-10).

For these reasons we have concluded that an in vivo assay system is still the most appropriate preparation in which to study the control of hepatic cholesterogenesis. Such a system is clearly complex, however, since one must deal with other variables such as diurnal rhythms in lighting that are known to also affect the rate of cholesterol synthesis (1, 11, 12). Recently, we have characterized such an assay system in the rat in detail using unfractionated intestinal lipoproteins (4). In these studies we showed that only liver manifested net cholesterol uptake after the intravenous injection of intestinal lipoproteins and, further, that this uptake was accounted for essentially entirely by an increase in cellular cholesterol ester content. The quantitative aspects of the relationship of net cholesterol uptake by the liver and the degree of inhibition of hepatic cholesterogenesis also was shown to be a complex function of when the lipoprotein was administered with respect to the diurnal rhythm of HMG-CoA reductase and whether the lipoproteins were administered as a single bolus or as a continuous infusion. Based on these studies, then, we have chosen two different types of in vivo assay procedures to examine the capacity of six different lipoprotein fractions obtained from intestinal lymph and serum of the rat to inhibit the rate of hepatic cholesterogenesis.

**EXPERIMENTAL PROCEDURE**

**Preparation of Lipoprotein Fractions from Intestinal Lymph and Serum.—**As previously described in detail, intestinal lymph was collected from donor rats fed a high cholesterol, high fat diet in flasks containing EDTA, penicillin G, and gentamycin (4). Every 12 hours the flasks were changed and the lymph was stored at 4°C. In order to study the metabolic effects of intestinal lipoproteins of different sizes and relative compositions, the whole intestinal lymph was separated arbitrarily into three fractions having different flotation numbers. For these separations the density was maintained at 1.006 and three separate fractions were harvested sequentially, using the three conditions of centrifugation shown in Table I. The S, values for these fractions were calculated as described in Ref. 13. Serum lipoprotein fractions were obtained from rats on two types of diets. In one set of studies large donor rats were fed ground Formulab chow diet containing 10% corn oil and 1% cholesterol for 1 week. Their blood was then sampled and, following surgery the rat was placed in an individual restraining cage and immediately returned to the light-cycling chamber where it was allowed free access to water and to the Formulab chow diet. Exactly 12 hours later at the mid-dark phase of the light cycle the assay rats were killed and liver slices were prepared. In a second group of studies the various lipoprotein fractions were administered to the recipient animals as a single bolus. In this assay procedure each animal was removed from the cycling chamber at the mid-light phase of the light cycle and fitted with a tail vein catheter. The animal was then kept in a restraining cage for approximately 20 min during which time it was infused with one of the lipoprotein fractions containing an appropriate amount of cholesterol or with an equal volume of 0.9% NaCl solution (control animals). Following completion of the bolus injection the catheter was removed immediately and the animal was returned to the light-cycling chamber and allowed continued access to water and to the Formulab chow diet. Exactly 12 hours later at the mid-dark phase of the light cycle the assay rats were killed and liver slices were prepared. In a second group of studies the various lipoprotein fractions were administered to the recipient animals as a single bolus. In this assay procedure each recipient animal was removed from the light-cycling chamber at the mid-dark phase of the light cycle and was fitted with an indwelling gastric and intravenous catheter (15). Following surgery the rat was placed in an individual restraining cage and immediately returned to the light-cycling chamber where it was allowed free access to water and was administered continuously through the gastric cannula a semisynthetic diet containing 45 g of casein, 105 g of dextrin, and 175 ml of water at a rate of 1.0 ml hour⁻¹ (15). In addition, the assay animal was also continuously infused intravenously with an appropriate amount of one of the lipoprotein fractions or with 0.9% NaCl solution (controls) at a rate of 1.0 ml hour⁻¹. Twenty-four hours later, at the mid-dark phase of the light cycle, the animal was killed and liver slices were prepared.

**Incubation Techniques and Chemical Procedures—**At the termination of both assay procedures the animals were killed by decapitation. The liver was removed, sliced, and incubated with [1-14C]jactonate as described in detail elsewhere (16). At the end of the incubation, the rates of incorporation of this radio-labeled precursor into cholesterol, CO₂, β-hydroxybutyrate, and acetocacetate were determined. In addition, the rates of synthesis of total ketones were measured and the specific activity of the newly synthesized ketones was calculated (16).

### Table I

**Characteristics of seven lipoprotein fractions used in this study**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Source of lipoproteins</th>
<th>Conditions of centrifugation</th>
<th>Total lipids (mg) (mg total cholesterol⁻¹)</th>
<th>Serum Cholesterol (mg) cm⁻²</th>
<th>Total Cholesterol (mg)</th>
<th>Lipoprotein (mg) cm⁻²</th>
<th>Total Cholesterol (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₁ &gt; 8000</td>
<td>Intestinal lymph</td>
<td>16,000 rpm x 10 min</td>
<td>138 ± 7</td>
<td>138 ± 7</td>
<td>138 ± 7</td>
<td>138 ± 7</td>
<td>138 ± 7</td>
</tr>
<tr>
<td>S₁ 400-8000</td>
<td>Intestinal lymph</td>
<td>22,000 rpm x 90 min</td>
<td>88 ± 6</td>
<td>88 ± 6</td>
<td>88 ± 6</td>
<td>88 ± 6</td>
<td>88 ± 6</td>
</tr>
<tr>
<td>S₁ 20-400</td>
<td>Intestinal lymph</td>
<td>22,000 rpm x 24 hr</td>
<td>35 ± 3</td>
<td>35 ± 3</td>
<td>35 ± 3</td>
<td>35 ± 3</td>
<td>35 ± 3</td>
</tr>
<tr>
<td>d &lt; 1.006</td>
<td>Serum</td>
<td>50,000 rpm x 24 hr</td>
<td>17 ± 5</td>
<td>17 ± 5</td>
<td>17 ± 5</td>
<td>17 ± 5</td>
<td>17 ± 5</td>
</tr>
<tr>
<td>d 1.006-1.070</td>
<td>Serum</td>
<td>60,000 rpm x 24 hr</td>
<td>8 ± 1</td>
<td>8 ± 1</td>
<td>8 ± 1</td>
<td>8 ± 1</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>d 1.070-1.215</td>
<td>Serum</td>
<td>60,000 rpm x 24 hr</td>
<td>5 ± 2</td>
<td>5 ± 2</td>
<td>5 ± 2</td>
<td>5 ± 2</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>Whole serum</td>
<td>Lipoprotein</td>
<td>60,000 rpm x 24 hr</td>
<td>6 ± 2</td>
<td>6 ± 2</td>
<td>6 ± 2</td>
<td>6 ± 2</td>
<td>6 ± 2</td>
</tr>
</tbody>
</table>

The abbreviation used is: HMG-CoA reductase, β-hydroxy-β-methylglutaryl-CoA reductase.
**RESULTS AND DISCUSSION**

Previous work published from this laboratory has characterized the general features of the control of hepatic cholesterologenesis by intestinal lipoproteins (1, 4, 15). On the basis of this work we have selected two types of assay procedures to evaluate the inhibitory capacity of various lipoprotein fractions on hepatic cholesterologenesis. The types of assays utilized along with several important metabolic and physiological parameters found in the assay animals under the conditions of these studies are shown in Fig. 1. After 2.5 weeks of light cycling the rate of cholesterologenesis in the liver (A) shows the expected marked fluctuation in activity from the mid-light to mid-dark phases of the light cycle under circumstances where the C3 flux into CO₂ (B) and the rate of ketone synthesis (C) are essentially constant. While not shown on this diagram it should be emphasized that the levels of free and esterified cholesterol in the liver cell also do not change during the 24-hour light cycle.² Of particular importance is the observation that in these animals the output of cholesterol in the intestinal lymph (E) is relatively constant throughout the 24-hour period at about 0.3 mg hour⁻¹ although a modest increase to approximately 0.4 mg hour⁻¹ is seen at the end of the dark phase, 6 hours after the peak level of gastric contents occurs (D). From these studies where the animals were fed a low cholesterol chow diet it can therefore be calculated that approximately 3 to 4 mg of cholesterol presumably reach the circulation from the intestinal lymph per 100 g of rat weight per 24 hours.

The time of injection of the various lipoprotein fractions relative to baseline cholesterogenic activity in the two types of assay procedures utilized in these studies is also shown at the

²Hepatic cholesterologenesis is known to be altered by a variety of dietary and environmental factors (1). In both published (4) and unpublished observations in this laboratory we have found that changes in the rate of cholesterol synthesis by the liver brought about by light cycling, stress, fasting, and interruption of the enterohepatic circulation of bile acids are not associated with changes in the content of cholesterol esters in the hepatocyte. In contrast, cholesterol feeding, bile acid feeding, and the intravenous administration of lipoproteins invariably are found to elevate the level of cholesterol esters in the liver.

### TABLE II

**Correlation of hepatic cholesterol ester content and rates of cholesterogenesis to amount of cholesterol carried in different lipoprotein fractions and injected intravenously into assay animals as single bolus**

<table>
<thead>
<tr>
<th>Lipoprotein fraction administered</th>
<th>A. n</th>
<th>B. Hepatic cholesterol esters/cholesterol input (mg g⁻¹)</th>
<th>C. In cholesterol synthesis/cholesterol input (mg g⁻¹⁻¹)</th>
<th>D. In cholesterol synthesis/hepatic cholesterol esters (mg g⁻¹⁻¹)</th>
<th>E. Mean animal weight (g)</th>
<th>F. Mean liver weight (g)</th>
<th>G. Cholesterol administered in liver (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₄ &gt; 8000</td>
<td>40</td>
<td>+0.108 ± 0.011</td>
<td>-0.106 ± 0.025</td>
<td>-1.02 ± 0.17</td>
<td>205 ± 6</td>
<td>7.5 ± 0.1</td>
<td>39.5</td>
</tr>
<tr>
<td>S₄ 400–8000</td>
<td>23</td>
<td>+0.071 ± 0.012</td>
<td>-0.097 ± 0.011</td>
<td>-1.31 ± 0.16</td>
<td>192 ± 7</td>
<td>7.2 ± 0.2</td>
<td>26.6</td>
</tr>
<tr>
<td>S₃ 30–400</td>
<td>36</td>
<td>+0.031 ± 0.006</td>
<td>0.112 ± 0.027</td>
<td>1.78 ± 0.41</td>
<td>106 ± 10</td>
<td>6.0 ± 0.3</td>
<td>10.9</td>
</tr>
<tr>
<td>Whole serum lipoproteins</td>
<td>14</td>
<td>+0.002 ± 0.003</td>
<td>-0.035 ± 0.015</td>
<td>-0.83 ± 0.51</td>
<td>201 ± 4</td>
<td>7.0 ± 6.0</td>
<td>0.7</td>
</tr>
</tbody>
</table>
carried in serum lipoproteins, hepatic cholesterol esters manifested essentially no increase in cholesterol ester content at the end of 12 hours. As seen in Panel B, the rate of synthesis of cholesterol by the liver was markedly suppressed by the injection of the lipoproteins from intestinal lymph but was much less inhibited by the injection of lipoproteins from serum. The slope of the regression curves indicated that the fractional rate of inhibition equaled 0.035 for each milligram of cholesterol injected in the serum lipoproteins but was nearly 3-fold greater (0.106) for cholesterol carried in the S₄ >8000 fraction. These alterations in cholesterol ester content and rate of cholesterol synthesis occurred under circumstances where the rate of hepatic ketone synthesis (F) and CO₂ flux into CO₂ (E) did not differ significantly from values found in control animals. Furthermore, the serum cholesterol levels had returned essentially to normal in both groups 12 hours after the bolus injections (D).

The slopes of the regression lines shown in Fig. 2 give the relationships between the amounts of cholesterol injected and the level of hepatic cholesterol esters (A) and rate of hepatic cholesterol synthesis (B), and between the level of hepatic cholesterol esters and the rate of cholesterol synthesis (C) and are entered in Table II along with similar data obtained after injection of two other fractions of intestinal lipoproteins having S₄ values of 400-8000 and 30-400. Three points merit emphasis concerning these data. First, as shown in Column B there are striking differences in the level of cholesterol esters achieved 12 hours after injection of the four different lipoprotein fractions: the incremental increase in esters was highest after injection of the large intestinal lipoproteins (0.108 mg g⁻¹) but progressively declined in value where the vehicle for the administration of the cholesterol was the S₄ 400-8000 (0.071 mg g⁻¹), S₄ 30-400 (0.031 mg g⁻¹), and serum (0.002 mg g⁻¹) lipoprotein fractions. Thus, as shown in Column G at the time the animals were killed 39.5% of the cholesterol administered in the S₄ >8000 fraction could be accounted for in hepatic cholesterol esters while, in contrast, only 0.7% of the cholesterol injected in serum lipoproteins was found in the liver. Second, the fraction rate of inhibition of hepatic cholesterol synthesis (Column C) was essentially the same for all three lipoprotein fractions obtained from lymph and varied from 0.097 to 0.112 while the degree of inhibition produced by whole serum lipoproteins was much less and equaled only 0.035. Third, the fractional rate of inhibition of cholesterol synthesis in the liver associated with an incremental increase in hepatic cholesterol esters of 1.0 mg g⁻¹ also varied with the particular lipoprotein fraction injected (Column D). This last result is similar to our previously reported finding where, after the injection of whole intestinal lymph lipoproteins, there was generally a correlation between inhibition of hepatic cholesterol synthesis and an increase in hepatocyte ester content but there was no constant quantitative relationship between these two variables (4). Thus, on the basis of these previous results as well as those of the present experiments, it appears that the decrement in cholesterol synthesis associated with a given increase in hepatocyte cholesterol ester content varies with the type of lipoprotein injected, the time frame of the experiment and whether the lipoprotein fraction was administered as a bolus or as a continuous infusion.

However, the values in Table II were all calculated from data points obtained 12 hours after injection of the lipoprotein fractions. In order to examine the possibility that there were marked differences in the clearance of these particles at shorter
FIG. 2. The rates of cholesterogenesis and other parameters of hepatic metabolism following the injection of a bolus of S₄ >8000 intestinal lipoproteins (LP) and whole serum lipoproteins. In these studies varying amounts of cholesterol carried in either the intestinal lymph lipoproteins or in serum lipoproteins were injected intravenously as a bolus at the mid-light phase into animals that had been subjected to light cycling for 2.5 weeks. All animals were then killed 12 hours later at the mid-dark phase of the cycle and various metabolic pathways were assayed in liver slices. In this diagram the level of hepatic cholesterol esters (A), rates of hepatic cholesterogenesis (B), CO₂ production (E), and ketone synthesis (F), and serum cholesterol levels (D) are plotted as a function of the amount of cholesterol administered in either of the lipoprotein fractions to the assay animals. In addition, the rate of hepatic cholesterogenesis is also plotted as a function of the level of cholesterol esters found in the liver at the time the animals were killed (C). The linear regression curves were fitted to the data by means of the method of least squares for the 40 animals injected with intestinal lipoproteins and the 14 animals injected with whole serum lipoproteins. The slopes of the lines in A, B, and C have been entered in Table II along with similar data for other lipoprotein fractions. The shaded area in each diagram represents the mean value ± 1 S.E. of each parameter found in 10 control animals injected with 0.9% NaCl solution.

time intervals, serum cholesterol levels and hepatic cholesterol ester concentrations were measured at frequent intervals after injection of amounts of the S₄ >8000, S₃ 30 to 400, and serum lipoprotein fractions that contained a constant load of total cholesterol equal to 6.1 mg 100 g⁻¹. These studies are shown in Fig. 3. The injection of all three fractions resulted in an identical rise in serum cholesterol levels immediately after completion of the injection at zero time to approximately 195 mg 100 ml⁻¹. As seen in Panel A, the smaller S₃ 30 to 400 lipoproteins were cleared rapidly from the blood so that the serum cholesterol level dropped to essentially normal levels within 3 hours after the injection. Coincident with this rapid clearance, as seen in Panel B, there was a prompt increase in hepatic cholesterol esters in the liver from 0.23 to 1.30 mg g⁻¹. The S₄ >8000 fraction behaved quite differently, however, for 120 min after injection of these particles the serum cholesterol level remained essentially constant at about 195 mg 100 ml⁻¹ and there was very little change in the hepatic cholesterol ester content. Beyond this time the serum cholesterol rapidly fell and there was a corresponding abrupt increase in the level of cholesterol esters in the liver. The serum lipoproteins were cleared more slowly than either of these intestinal fractions and produced an increment in the ester content of the liver of only approximately 0.4 mg g⁻¹.

In addition to these differences in rates of uptake, there were also differences evident in the rates of clearance of cholesterol esters from the liver. For example, the cholesterol ester level in the liver reached similar values after injection of both the S₄ >8000 and S₃ 30 to 400 fractions but at both 12 and 24 hours the ester content had declined much more in those animals injected with the S₃ 30 to 400 fraction than in those injected with the large intestinal lipoprotein particles. Thus, the incremental changes in cholesterol esters found with the various lipoprotein fractions and given in Column B of Table II are a complex function of the rate of uptake by the liver of cholesterol carried in these different fractions as well as the rate of hepatic disposal of cholesterol esters. Both of these processes are affected by the type of lipoprotein injected.

These data provide the basis for a more detailed analysis of the relationship of cholesterol ester levels in the hepatocyte to the rate of cholesterol synthesis. Several lines of evidence now suggest that the increase in HMG-CoA reductase activity seen between the mid-light and mid-dark phases of the light cycle is due to synthesis of new enzyme protein (20, 21). Assuming that some fraction of the cellular cholesterol ester pool operates as the effector in inhibiting the rate of cholesterol synthesis and, further, that HMG-CoA reductase is equally sensitive to regulation throughout the 12-hour period during which these assays were carried out, then there should be a direct relationship between the amount of inhibition observed and the level of
That this is the case is strongly suggested by the finding that the ratios of the areas under the three curves shown in Panel B, Fig. 3 (S, >8000 (1.00): S, 30 to 400 (1.11): serum lipoproteins (0.34)) are essentially identical with the ratios of the fractional rates of inhibition (1.00/0.60/0.33) listed in Column C of Table II for the three lipoprotein fractions. Thus, under the precisely controlled conditions of this assay procedure the amount of inhibition of the cholesterogenic pathway appears to be determined directly by the degree of elevation of the cholesterol ester content produced by a given lipoprotein fraction and the time course over which this elevation takes place.

Another conclusion of physiological importance to be drawn from these studies is that serum lipoproteins appear to be relatively ineffective regulators of hepatic cholesterogenesis. However, in the studies shown in Fig. 3 and Table II the serum had been harvested from rats fed a low cholesterol, low fat chow diet where, as described in detail by Lasser et al. (22) and confirmed in this laboratory, the great majority of the serum cholesterol is carried in high density lipoproteins and there is relatively little cholesterol in the fractions with densities <1.070. In order to test the possibility, therefore, that one of these lower density serum lipoprotein fractions might have much higher inhibitory activity a large volume of rat serum was processed to yield sufficient amounts of lipoproteins with densities of <1.006 and 1.006 to 1.070 to inject three animals with each of these fractions: the fractional rate of inhibition averaged 0.031 for the former and 0.025 for the latter. While the number of animals in this experiment is too small for statistical analysis, the results are consistent with those found after injection of the whole serum lipoprotein fraction and suggest that all of the major serum lipoproteins are much less effective as inhibitors of hepatic cholesterogenesis than are the lipoproteins of intestinal origin.

We next turned to the second type of assay procedure in order to test the regulatory capacity of these different lipoprotein fractions under the more physiological circumstance where they were administered in low concentrations by continuous intravenous infusion for a 24-hour period. The same three fractions of intestinal lipoproteins were utilized in these studies. However, the serum lipoproteins were obtained from rats fed a high cholesterol, high fat diet in order to increase the amount of cholesterol carried in the lower density fractions so that sufficient quantities of cholesterol in serum lipoproteins with densities of <1.006 and 1.006 to 1.070 as well as 1.070 to 1.215 could be obtained to test in these assays.

These studies, summarized in Table III, again show significant differences between the ability of the six lipoproteins tested to cause net increases in cholesterol ester content in the liver and to inhibit the rate of hepatic cholesterol synthesis. As shown in Column B, the observed increase in cholesterol esters was greatest with the S, >8000 fraction (0.053) and decreased as the intestinal lipoprotein fractions with S, values of 400 to 8000 (0.014) and 30 to 400 (0.004) were injected. Furthermore, all three serum lipoprotein fractions were much less effective in causing a net increase in cholesterol ester content than the S, >8000 intestinal lipoprotein fraction, and, because of variation in the data, no significant difference was evident among these three fractions. At the time the animals were killed 19.9% of the administered dose of cholesterol could be accounted for in the ester fraction in the livers of the animals infused with the S, >8000 fraction whereas significantly lesser amounts were present after injection of the other fractions. These particular results are qualitatively similar to those obtained with the bolus injection assays (Table II). As seen in Column C, however, the fractional rate of inhibition was nearly 6-fold greater when the S, >8000 intestinal lipoproteins were infused than when the S, 30 to 400 fractions were administered. However, since the incremental increase in cholesterol esters was disproportionately lower (Column B) for the S, 400 to 8000 and S, 30 to 400 fractions than the fractional rates of inhibition (Column C), the amount of inhibition manifested per mg of incremental increase in cholesterol esters increased from 1.17 (S, >8000) to 4.21 (S, 30 to 400) as shown in Column D. Again, the three serum lipoprotein fractions were able to inhibit hepatic cholesterogenesis but at rates that were only about 14 to 35% of that seen with the S, >8000 intestinal lipoproteins, and, as with the bolus injections, there was no significant

**Fig. 3.** Detailed time courses for the clearance of serum cholesterol and the change in hepatic cholesterol ester levels after the administration of bolus of either S, >8000 or S, 30 to 400 intestinal lipoproteins or whole serum lipoproteins. All animals in this study were subjected to light cycling for 3 weeks prior to use. At the mid-light phase of the cycle each animal was then administered a bolus of one of these three lipoprotein fractions containing 5.9 to 6.2 mg 100 g⁻¹ of total cholesterol; control animals were injected with an equal volume of 0.9% NaCl solution. Groups of such injected animals were then killed at frequent intervals for up to 24 hours thereafter for determination of serum cholesterol and hepatic cholesterol ester levels. The shaded areas in the diagram represent the mean values ± 1 S.E. for these two parameters measured in 10 control animals killed throughout the 24-hour period of observation. The other points are mean values ± 1 S.E. for 4 to 10 animals in each group.

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All assay animals used in this study were subjected to light cycling for 2.5 weeks prior to use. Each animal was then fitted with an indwelling gastric and intravenous cannula at the mid-dark phase of the light cycle. The rats were then returned to the light-cycling chamber and infused continuously through the intravenous cannula with one of six different lipoprotein fractions containing varying amounts of cholesterol: in addition, the animals also received a semi-synthetic diet continuously through the gastric cannula at a rate of 1.0 ml/hour. Twenty-four hours later at the mid-dark phase of the light cycle the animals were killed and various parameters of liver metabolism were assayed. The data in each column were derived as described in Table II.

### Table III

**Correlation of hepatic cholesterol ester content and rates of cholesterogenesis to amount of cholesterol carried in different lipoprotein fractions and administered as continuous intravenous infusion for 24 hours**

<table>
<thead>
<tr>
<th>Lipoprotein fraction administered</th>
<th>A. n</th>
<th>B. Hepatic cholesterol esters/cholesterol input (mg g⁻¹)</th>
<th>C. In cholesterol synthesis/cholesterol input (mg 100 g⁻¹)</th>
<th>D. In cholesterol synthesis/hepatic cholesterol esters (mg⁻¹)</th>
<th>E. Mean animal weight (g)</th>
<th>F. Mean liver weight (g)</th>
<th>G. Cholesterol administered in liver (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₁ &gt;8000</td>
<td>9</td>
<td>+0.050 ± 0.014</td>
<td>−0.084 ± 0.015</td>
<td>−1.17 ± 0.31</td>
<td>192 ± 6</td>
<td>7.2 ± 0.2</td>
<td>19.9</td>
</tr>
<tr>
<td>S₁ 400-800</td>
<td>10</td>
<td>+0.014 ± 0.004</td>
<td>−0.040 ± 0.018</td>
<td>−1.95 ± 0.80</td>
<td>201 ± 5</td>
<td>6.9 ± 0.1</td>
<td>4.8</td>
</tr>
<tr>
<td>S₁ 30-400</td>
<td>10</td>
<td>+0.004 ± 0.002</td>
<td>−0.013 ± 0.005</td>
<td>−4.21 ± 1.01</td>
<td>196 ± 2</td>
<td>7.2 ± 0.3</td>
<td>1.5</td>
</tr>
<tr>
<td>d &lt;1.006</td>
<td>13</td>
<td>+0.003 ± 0.003</td>
<td>−0.021 ± 0.015</td>
<td>−1.30 ± 0.15</td>
<td>204 ± 7</td>
<td>7.4 ± 0.2</td>
<td>1.1</td>
</tr>
<tr>
<td>d 1.006-1.070</td>
<td>11</td>
<td>+0.009 ± 0.005</td>
<td>−0.012 ± 0.006</td>
<td>−0.73 ± 0.25</td>
<td>189 ± 5</td>
<td>6.7 ± 0.3</td>
<td>3.2</td>
</tr>
<tr>
<td>d 1.070-1.915</td>
<td>9</td>
<td>+0.014 ± 0.010</td>
<td>−0.000 ± 0.015</td>
<td>−0.77 ± 0.12</td>
<td>194 ± 3</td>
<td>6.8 ± 0.2</td>
<td>1.9</td>
</tr>
</tbody>
</table>

**Table III**

*Correlation of hepatic cholesterol ester content and rates of cholesterogenesis to amount of cholesterol carried in different lipoprotein fractions and administered as continuous intravenous infusion for 24 hours*

<table>
<thead>
<tr>
<th>Correlation of hepatic cholesterol ester content and rates of cholesterogenesis to amount of cholesterol carried in different lipoprotein fractions and administered as continuous intravenous infusion for 24 hours.</th>
</tr>
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<tbody>
<tr>
<td>All assay animals used in this study were subjected to light cycling for 2.5 weeks prior to use. Each animal was then fitted with an indwelling gastric and intravenous cannula at the mid-dark phase of the light cycle. The rats were then returned to the light-cycling chamber and infused continuously through the intravenous cannula with one of six different lipoprotein fractions containing varying amounts of cholesterol: in addition, the animals also received a semi-synthetic diet continuously through the gastric cannula at a rate of 1.0 ml/hour. Twenty-four hours later at the mid-dark phase of the light cycle the animals were killed and various parameters of liver metabolism were assayed. The data in each column were derived as described in Table II.</td>
</tr>
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</table>

It is evident that the results obtained in these constant infusion assays differed from those obtained with a bolus injection (Table II) in one important respect. In the bolus injection experiments the fractional rate of inhibition was the same with the three intestinal lipoprotein fractions while in the 24-hour infusion studies the amount of inhibition produced varied directly with the S₁ value for the particle and, therefore, with the size of the lipoprotein and the amount of non-sterol lipid administered to the animals (Table I). This finding is consistent with, and emphasizes the importance of, the observation shown in Fig. 3 that disposition of hepatic cholesterol esters is less rapid in animals infected with the S₁ >8000 intestinal lipoprotein fraction. Presumably, in the constant infusion assays the administration of the large intestinal lipoproteins results in a “steady state” level of cholesterol esters that is higher than that obtained with the other fractions and that, in turn, results in a greater degree of inhibition of hepatic cholesterogenesis per mg of cholesterol administered.

Unlike the acute bolus injection experiments, however, it is impossible to quantitate this relationship since complete data on the level of hepatic cholesterol esters throughout the 24-hour period of infusion are not available in these technically difficult experiments. Furthermore, as shown in Fig. 1, the base-line of cholesterol synthesis varies markedly during this 24-hour period, and the sensitivity of the rate-limiting enzyme in the cholesterogenic pathway to the levels of hepatic cholesterol esters at each of these different time periods is currently unknown.

Finally, four additional points concerning these experiments warrant comment. First, no attempt was made in these studies to determine whether free cholesterol or esterified cholesterol, or both, was the chemical species taken up from the lipoprotein into the hepatocyte. Since the proportion of cholesterol esters in all six lipoprotein fractions tested in these studies was essentially the same, equaling approximately 63 to 72% of the total cholesterol in any fraction, differences in ester content cannot explain the observed differences in uptake rates. Furthermore, since nearly all of the cholesterol carried in the intestinal lipoproteins is apparently taken up into the liver, it is also apparent that both free and esterified cholesterol must reach the cytosol of the hepatocyte. Whether or not the cholesterol esters are hydrolyzed first prior to membrane translocation is currently unknown: however, once inside the cell essentially all of the absorbed sterol is stored temporarily as esters.

Second, these studies can also be related to several aspects of sterol balance in the intact animal. For example, Wilson has shown in the baboon that there is essentially a quantitative inverse relationship between the amount of dietary cholesterol absorbed and the rate of de novo cholesterol synthesis in the liver (23). Dietary cholesterol reaches the circulation only in lipoproteins of intestinal origin and, as shown in these studies, the cholesterol carried in this specific fraction is selectively, rapidly, and essentially completely taken up into the liver before being disposed of elsewhere. Thus, it is apparent that this system is uniquely suited to tightly coordinating the rate of cholesterol synthesis in the liver to the amount of cholesterol absorbed from the intestine. Similarly, there are also other data supporting the concept that various serum lipoprotein fractions play only a minor role, in regulating sterol synthesis in the liver. Hepatic cholesterol synthesis, for example, is not suppressed in various hypercholesterolemic states (24, 25) and in biliary obstruction where the serum cholesterol may be grossly elevated there is actually a 2- to 3-fold increase in the rate of cholesterol synthesis. 

A third major conclusion that is supported by these studies is that within these serum lipoprotein fractions...
that the amount of inhibition of hepatic cholesterol genesis is correlated closely with the cholesterol ester level achieved in liver cells after administration of a particular lipoprotein fraction. This correlation is seen in general terms in our previously reported studies in which the time frame and mode of administration of unfractionated intestinal lipoproteins was varied (4) and in the present study, particularly those experiments involving the 24-hour constant infusion assay (Table III). However, the strongest support for this conclusion comes from the bolus injection studies (Fig. 3) where the fractional rate of inhibition can be related quantitatively to the concentration of cellular cholesterol ester integrated as a function of time. This finding, in turn, implies that other components of the lipoprotein, e.g., the specific peptide chains, play no direct role in the inhibitory process. This correlation, however, does not necessarily imply that cholesterol esters are the feedback effector within the cell. It is still possible that the actual effector is a small, undetected pool of unesterified cholesterol or even some unrecognized metabolic product of cholesterol.

Fourth, on the other hand, the characteristics of specific lipoproteins in which cholesterol is delivered to the liver do, indirectly, influence the rate of sterol synthesis insofar as they apparently determine the level of cholesterol esters achieved in the liver in a particular experimental setting. The experimental results obtained with the 12-hour bolus injection indicate that the differences observed in this regard with the various lipoprotein fractions must be explained in terms of two separate processes: (a) the rate at which the cholesterol is taken up by the liver from each of the lipoprotein fractions and (b) the rate at which cholesterol ester is disposed of by the liver. That the rate of net cholesterol uptake is strikingly different from the different fractions is shown in Panel B of Fig. 3: net uptake rates calculated during the first 80 min after injection, for example, equaled 0.0024 (mg g⁻¹) (mg 100 g⁻¹)⁻¹ (hour)⁻¹ from the S₃ >8000 particle but were 12.5 times greater for the S, 30 to 400 particle equaling 0.030 (mg g⁻¹) (mg 100 g⁻¹)⁻¹ (hour)⁻¹. These rate constants correspond to a velocity of net sterol uptake equal to 0.9 and 11.0% of the administered load of lipoprotein cholesterol, respectively, and can be compared to a rate of uptake of 3.8% of the cholesterol load administered as uncomplexed, whole intestinal lymph lipoprotein previously reported (4). It is also apparent in Fig. 3 that in the case of the S₃ >8000 particle the uptake rate suddenly increased after 80 to 100 min presumably as a consequence of metabolic alteration of the large, triglyceride-rich particles in the periphery (29). There are essentially no transport data yet available to explain these marked variations in cholesterol uptake rates by the liver. If a finite number of recognition sites on the sinusoidal membrane are required for this process, then the sites must be relatively nonspecific since it is clear from these studies that all classes of lipoprotein are capable of transferring cholesterol to the liver, albeit at markedly different rates. Alternatively, uptake may occur through some process such as direct partitioning between the lipoprotein and the sinusoidal cell membrane or through an obligatory monomeric phase. If one of these latter possibilities is correct, then the differences in uptake rates must be explained in terms of differences in relative polarity of the lipoprotein fractions or in terms of differences in resistance encountered for molecular diffusion through unstirred layers in the space of Disse. The manner in which lipoproteins alter the rate of cholesterol ester disposition from the liver also is not understood. As seen in Table I, however, the higher the S₃ value the greater the amount of other lipids, principally triglyceride, carried in the fraction per mg of total cholesterol. This lipid or some other component may directly or indirectly slow the rate of movement of cholesterol ester from the liver into other lipoprotein classes or into bile acids. In any event, the elucidation of the reasons for this also is of considerable importance since this effect probably explains why in the continuous infusion studies the level of hepatic cholesterol esters and the degree of inhibition of cholesterol synthesis is directly related to the S₃ value of the fraction of intestinal lipoproteins injected (Table III).

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