The Kinetic Characteristics of Inhibition of Hepatic Cholesterogenesis by Lipoproteins of Intestinal Origin*

(Received for publication, August 19, 1974)

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SUMMARY

In these studies intestinal lipoproteins were injected intravenously into recipient rats in order to study the kinetic characteristics of cholesterol uptake by the liver cell and inhibition of the cholesterol synthetic pathway. Net cholesterol uptake from circulating intestinal lipoproteins took place only in the liver, and only this tissue manifested inhibition of cholesterol synthesis. Cholesterol uptake by the liver, quantified by a rise in the cholesterol ester content, was a linear function of time and of the amount of lipoprotein cholesterol administered to the animals. Using groups of rats that were either fed cholesterol or injected intravenously with intestinal lipoproteins as a bolus or as a continuous infusion, there was generally a correlation between inhibition of the rate of cholesterol synthesis and the cholesterol ester content of the liver. However, there was no consistent quantitative relationship between these two variables suggesting either that cholesterol ester was not the immediate effector of the inhibition or, alternatively, that there was intracellular localization of the effector at the site of control of the rate-limiting enzyme in the cholesterogenic pathway.

The control of the rate of synthesis of cholesterol in the liver is known to be unusually complex, since this metabolic pathway responds to such diverse physiological variables as total caloric intake, the cholesterol content of the diet, the size of the bile acid pool, stress, various hormones, and even diurnal variation in lighting (1–11). In each of these situations it is now fairly well established that variations in the rate of the over-all bio-

* This work was supported by United States Public Health Service Research Grant HL09610, United States Public Health Service Institutional Research Training Grant GM00034, and by a grant from the John and Mary Markle Foundation. During the period when these studies were being carried out, F. O. N. was partially supported by a grant from Catholic University of Chile, and H. J. W. was partially supported by Deutsche Forschungsgemeinschaft Grant We 410/2.

1 Unpublished observations made in this laboratory.
were added to each flask just prior to starting the collection. Every 12 hours the flasks were changed, and the lymph was stored at 4°C. The whole lymph was centrifuged for 4 hours at 22,000 rpm in an SW 25.1 swinging bucket rotor at 5°C, and the layer of lipoproteins was then re-suspended in a small volume of 0.9% NaCl solution to give a final concentration of cholesterol in the range of 3.5 to 5.0 mg/ml. This material was dialyzed against two changes of 0.9% NaCl solution at 4°C for 10 to 12 hours following which it was ready for injection into experimental animals. The amount of lipoprotein injected into an animal is expressed as the milligrams of total cholesterol contained in the lipoprotein fraction normalized to 100 g of animal weight, i.e. mg/100 g⁻¹. During the course of these studies 20 such batches of lymph were processed. In these, 63 ± 3% of the total cholesterol was present as cholesterol esters, and there were 107 ± 6 mg of total lipid/mg of total cholesterol (16).

**Animal Preparations**—Female, Sprague-Dawley-derived rats weighing 190 to 220 g were used as the recipient animals and were kept in light-tight chambers with forced air ventilation at 22 to 25°C for a minimum of 2.5 weeks. The lighting in these chambers was cycled through 12 hours of light and 12 hours of dark by automatic timers. Throughout this period of adaptation, the animals had free access to water and to Formulab Chow. After this initial period of adaptation to light cycling, three types of experimental animals were prepared.

First, a group of animals was prepared with indwelling gastric and intravenous cannulas and, in addition, some animals also were subjected to biliary diversion, biliary obstruction, or intestinal lymphatic diversion (14). After surgery all animals were placed in individual restraining cages and immediately returned to their appropriate light-cycling chambers. They were allowed free access to water and were infused continuously at a rate of 1.0 ml/hour through the gastric cannula with a semisynthetic diet containing 45 g of casein, 105 g of dextrin, and 175 ml of water (pH 7.0) (14). In certain experiments (see Table I) either sodium taurocholate or cholesterol was mixed into this diet using a Food blender. In other experiments, animals were continuously infused through the intravenous catheter with lipoproteins. Regardless of treatment, these animals were all killed after 48 hours. A second group of studies was undertaken where intestinal lipoproteins were injected intravenously into recipient animals as a single bolus. These rats were quickly removed from the light-cycling chambers and brieﬂy anesthetized with diethyl ether: a PE 10 polyethylene catheter was introduced into a tail vein, and the animals were then placed in individual restraining cages. A total volume of 5 ml of either 0.9% NaCl solution or the lipoprotein suspension was then injected by means of an infusion pump (B. Braun, Melsungen, West Germany) at a rate of 0.2 ml/min. After completion of the injections, the tail vein catheters were removed, and the animals were returned to the light-cycling chambers for varying periods of time until they were killed. In a third type of study, animals were placed in individual metabolic cages in light-cycling chambers and fed 18 g of ground rat chow containing 0, 0.1, 0.3, 0.8, or 1.2% cholesterol each day for 48 hours, following which they were killed.

**Incubation Techniques and Chemical Procedures**—At the termination of all in vivo manipulations, the animals were killed by decapitation. The livers were removed, sliced, and incubated with [1-14C]octanoate as described in detail elsewhere (17). At the end of the incubation, the rates of incorporation of this radiolabeled precursor into cholesterol, CO₂, ß-hydroxybutyrate, and acetacetate were determined. In addition, the rates of synthesis of total ketones were measured, and the specific activity of the newly synthesized ketones was calculated (17). The incorporation rates of [1-14C]octanoate into cholesterol were then corrected for intramitochondrial dilution of the specific activity of the acetyl-CoA pool from the oxidation of endogenous substrates using the specific activity of the total ketones (17, 18). Rates of cholestero genesis were calculated as the nanomoles of acetyl-CoA, i.e., C₂ units, incorporated into cholesterol per g wet weight of liver slices per hour of incubation, i.e., nanomoles-g⁻¹·hour⁻¹. Aliquots of blood, various tissues, and the lipoprotein fraction injected were also obtained for determination of total cholesterol and cholesterol ester content (16, 19, 20).

**Mathematical Treatment of Data**—Where appropriate, mean values for groups of data are given ± S.E. For time courses and concentration courses, linear regression curves were fitted to the data obtained from individual animals, and have the usual form of \( y = a + bx \), where \( a \) equals the intercept on the y axis, and \( b \) is the proportionality constant between the two variables \( x \) and \( y \). The values of \( a \) and \( b \) are given along with ±1 S.D. As has been reported before for cholesterol feeding (13) and for lipoprotein injection (14), the rate of hepatic cholesterol synthesis appears to vary in a log-linear fashion to the amount of cholesterol administered to the animal or to the hepatic cholesterol ester content. Hence, in studies such as those shown in Fig. 3, the natural logarithm, \( \ln \), of the C₂ flux into cholesterol was used for regression analysis of the relationship between the rate of cholesterol synthesis and cholesterol intake and hepatic cholesterol ester content.

### Table 1

**Effect of various manipulations of enterohepatic and enterolymphatic circulation on hepatic cholesterol content**

<table>
<thead>
<tr>
<th>Animal preparation</th>
<th>Experimental manipulation</th>
<th>Hepatic cholesterol content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total cholesterol</td>
</tr>
<tr>
<td>1. Control</td>
<td>0 Cholesterol diet</td>
<td>2.12 ± 0.15</td>
</tr>
<tr>
<td>2. Biliary fistula</td>
<td>0 Cholesterol diet</td>
<td>1.94 ± 0.08</td>
</tr>
<tr>
<td>3. Biliary obstruction</td>
<td>0 Cholesterol diet</td>
<td>2.25 ± 0.21</td>
</tr>
<tr>
<td>4. Lymphatic fistula</td>
<td>0.1% Taurocholate diet</td>
<td>1.90 ± 0.08</td>
</tr>
<tr>
<td>5. Control</td>
<td>0.3% Cholesterol diet</td>
<td>3.28 ± 0.45</td>
</tr>
<tr>
<td>6. Control</td>
<td>1.0% Cholesterol diet</td>
<td>5.52 ± 0.32</td>
</tr>
<tr>
<td>7. Control</td>
<td>IV Intestinal LP</td>
<td>3.15 ± 0.25</td>
</tr>
<tr>
<td>8. Biliary fistula</td>
<td>IV Intestinal LP</td>
<td>3.29 ± 0.45</td>
</tr>
<tr>
<td>9. Biliary obstruction</td>
<td>IV Intestinal LP</td>
<td>3.92 ± 0.61</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Previous work has shown that hepatic cholesterol esters increase with uptake of cholesterol by the liver; however, it has also been postulated that the concentration of esters in the liver may increase if the degradation of cholesterol to bile acids is inhibited (21). Initial studies, therefore, were undertaken to evaluate this possibility. As shown in Lines 2 and 3 of Table I, the content of cholesterol esters in the liver of animals with biliary diversion, where cholesterol degradation to bile acids is increased (6), and in biliary obstruction, where bile acid synthesis is suppressed (22, 23), was the same as in control animals. Furthermore, when bile acids were fed to animals with intestinal lymphatic diversion to suppress bile acid synthesis under circumstances where delivery of cholesterol from the intestine to the liver was interrupted, the content of cholesterol esters in the liver again was found to be normal (Lane 4). In contrast, feeding cholesterol (Lines 5 and 6) or the administration of cholesterol intravenously in the form of intestinal lipoproteins (Lines 7, 8, and 9) markedly elevated hepatic cholesterol ester content. It is also evident in Table I that increases in the total cholesterol content of the liver in the various experimental manipulations resulted almost entirely from an increase in the cholesterol ester content: the levels of unesterified cholesterol remained essentially constant at 1.5 to 1.9 mg g⁻¹. We have concluded from these observations, therefore, that the concentration of hepatic cholesterol esters primarily reflects the rate of net cholesterol uptake by the hepatocytes and not the rate of either de novo cholesterol synthesis or cholesterol degradation to bile acids by the liver.

The specificity of this uptake step was next evaluated. As shown in Table II, after the injection of a bolus of lipoproteins containing 9.5 mg · 100 g⁻¹ of cholesterol, both the content of total cholesterol and cholesterol esters increased in liver. No other tissue showed a significant difference between the control and the injected groups. Hence, extrahepatic tissues that fail to respond to cholesterol feeding with suppression of de novo cholesterol synthesis (24) also fail to manifest a net increase in cholesterol content when exposed to circulating intestinal lipoproteins. This specificity may imply the presence of specific recognition sites on the sinusoidal membrane of the hepatocyte for the lipoprotein. Alternatively, however, it may only indicate specificity of some intracellular metabolic event such as esterification. For example, the unidirectional flux of unesterified cholesterol from the lipoprotein might occur equally well into all tissues but only the liver may immediately esterify the cholesterol, preventing an equal but oppositely directed backflux out of the cell. Which of these processes confers specificity for uptake of cholesterol by the liver cannot be determined on the basis of available data.

In order to study the kinetic relationships between cholesterol uptake by the liver and several parameters of hepatic cholesterol metabolism, two different experimental approaches were utilized. In the first type the lipoproteins were injected intravenously, rapidly, as a single bolus, while in the second group of studies the lipoproteins were administered over a prolonged period of time as a continuous infusion. The results obtained from the first set of experiments using the bolus injection are shown in Figs. 1, 2, and 3.

Since the rate of hepatic cholesterogenesis in the rat is known to change markedly during diurnal variation in lighting, initial studies were undertaken by injecting a constant amount of lipoproteins 0.5, 6, 12, 24, and 48 hours prior to killing the animals at the mid-dark phase of the light cycle. As seen in Fig. 1, after injection of a bolus of lipoproteins containing 8.0 mg · 100 g⁻¹ of cholesterol, the serum cholesterol levels reached approximately 200 mg · 100 ml⁻¹ when the injection occurred 0.5 hours prior to the time the animals were killed, but were normal if the injection had occurred 12 hours or longer prior to termination of the experiment. Coincident with the rapid clearance of circulating serum cholesterol there was a rise in cholesterol esters in the liver. At the point when the serum cholesterol levels became normal, hepatic cholesterol ester content began a slow decline reaching normal values only when the interval between the injection and termination of the experiment was 48 hours. The rate of hepatic cholesterol synthesis, shown in the lower panel, varied inversely with the level of hepatic cholesterol esters. Again, fully 48 hours were required for the rate of synthesis to return to control levels after the bolus injection.

A more detailed examination of the clearance of circulating cholesterol and uptake by the liver during the first 120 min after

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Total cholesterol content</th>
<th>Cholesterol ester content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>LP injected</td>
</tr>
<tr>
<td>Liver</td>
<td>2.00 ± 0.10</td>
<td>3.93 ± 0.13</td>
</tr>
<tr>
<td>Stomach</td>
<td>2.33 ± 0.14</td>
<td>2.47 ± 0.23</td>
</tr>
<tr>
<td>Jejunum</td>
<td>1.57 ± 0.13</td>
<td>1.73 ± 0.03</td>
</tr>
<tr>
<td>Colon</td>
<td>1.40 ± 0.06</td>
<td>1.53 ± 0.03</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.43 ± 0.03</td>
<td>3.55 ± 0.15</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>0.57 ± 0.09</td>
<td>0.53 ± 0.09</td>
</tr>
<tr>
<td>Lung</td>
<td>3.20 ± 0.11</td>
<td>3.26 ± 0.09</td>
</tr>
<tr>
<td>Cardiac muscle</td>
<td>1.27 ± 0.03</td>
<td>1.23 ± 0.03</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0.57 ± 0.03</td>
<td>0.53 ± 0.03</td>
</tr>
<tr>
<td>Skin</td>
<td>2.27 ± 0.33</td>
<td>2.13 ± 0.03</td>
</tr>
<tr>
<td>Brain</td>
<td>14.80 ± 1.10</td>
<td>12.03 ± 0.50</td>
</tr>
</tbody>
</table>

Animals that had been subjected to light cycling for 3 weeks were administered either 0.9% NaCl solution or a bolus of intestinal lipoproteins (LP) containing 9.5 mg of cholesterol/100 g of animal weight at the mid-light phase of the light cycle. Twelve hours later at the mid-dark phase of the cycle they were killed for three animals in each group.
this time interval, as seen in the left panel, there was only a slight decline in the serum cholesterol level which was accompanied by a similar gradual, but significant, increase in the hepatic cholesterol ester content. When varying amounts of lipoproteins were injected in a second group of rats that were all killed 80 min later, as seen in the right panel, the cholesterol ester level in the liver increased by 0.015 mg·100 g⁻¹ for each mg·100 g⁻¹ of cholesterol administered in the lipoprotein fraction.

As shown in Fig. 1, maximum inhibition of cholesterol synthesis was found when the bolus of lipoproteins was injected 12 hours prior to killing the animals. Therefore, studies designed to evaluate the kinetic relationships between the amount of cholesterol injected and several parameters of hepatic cholesterol metabolism were next undertaken by injecting varying amounts of intestinal lipoproteins 12 hours prior to killing the experimental animals at the mid-dark phase of the light cycle. As is apparent in the first panel of Fig. 3, in the 20 animals used in this study the level of hepatic cholesterol esters increased linearly with the amount of cholesterol administered so that esters rose by 0.009 ± 0.004 mg·100 g⁻¹ for each 1.0 mg·100 g⁻¹ of cholesterol injected. In contrast, the rate of cholesterol synthesis by the liver decreased in a log-linear relationship to both the amount of cholesterol administered and to the hepatic cholesterol ester content of the liver as shown in the middle and right panels, respectively. Synthesis decreased by a factor of 0.052 ± 0.009 for each 1.0 mg·100 g⁻¹ of cholesterol injected and by a factor of 0.78 ± 0.13 for an incremental increase of 1.0 mg·g⁻¹ in hepatic cholesterol ester content.

These latter studies were all undertaken using a single, rapid injection of the lipoprotein bolus so that a high concentration of circulating chylomicrons was obtained, which, during the subsequent period of observation, progressively fell as the lipoproteins were cleared from the vascular space. The relationship between the amount of lipoprotein cholesterol injected into the assay animals and the various parameters of cholesterol metabolism was next evaluated in the more physiological circumstance where the intestinal lipoproteins were administered intravenously as a continuous infusion for varying times during the experimental period. As seen in Fig. 4, the level of serum cholesterol and the hepatic cholesterol ester content varied with total duration of time over which a fixed amount of lipoprotein cholesterol was infused: for example, when 23 mg·100 g⁻¹ was infused continuously during the 12 hours prior to killing the animals, the mean serum cholesterol and hepatic cholesterol ester level equalled 265 ± 25 mg·100 ml⁻¹ and 2.8 mg·g⁻¹, respectively. However, when this same amount of cholesterol was infused over a 48-hour period, the steady state serum cholesterol level was essentially normal, and the hepatic cholesterol ester level increased to only 0.42 mg·g⁻¹. Similar findings were seen with infusion of the lower dose of 8 mg·100 g⁻¹ of cholesterol. Despite the marked dependency of these two parameters on the time interval over which the lipoprotein cholesterol was administered, as seen in the lower panel, essentially identical amounts of inhibition of hepatic cholesterogenesis were found with a given dose of cholesterol regardless of the period over which the infusion took place.

Finally, in order to obtain the quantitative relationships between the amount of cholesterol continuously infused and the level of hepatic cholesterol esters and rate of cholesterogenesis, as was done in the case of the bolus injections (Fig. 3), a series of animals were infused continuously for either 24 or 48 hours with varying amounts of lipoprotein cholesterol. The results of these studies are summarized in Lines 3 and 4 of Table III. For comparison, the data obtained from the animals injected with a bolus of lipoproteins 12 hours prior to killing (Fig. 3) are shown in Line 2, while the values in Line 1 were derived from another group of animals that were fed, isocalorically, a diet containing varying amounts of cholesterol over a 48-hour period.
Fig. 3. Effect of injecting varying amounts of intestinal lipoproteins (LP) as a bolus 12 hours prior to killing the animals on hepatic cholesterol ester content and rate of hepatic cholesterol synthesis. Animals adapted to light cycling for 2.5 to 3 weeks were injected with varying amounts of intestinal lipoproteins as a bolus at the mid-light phase of the light cycle and were killed 12 hours later at the mid-dark phase of the cycle. The amount of cholesterol contained in the lipoproteins varied from 0.9 to 29.5 mg/100 g of animal weight. In this diagram three correlations are shown. In the left panel hepatic cholesterol ester content is plotted as a function of the amount of cholesterol injected in the intestinal lipoproteins. In the middle and right panels, respectively, the rate of C2 flux into cholesterol by liver slices is plotted semilogarithmically against the amount of cholesterol injected and the hepatic cholesterol ester content. The regression lines were fitted to the data obtained in 29 individual animals by the method of least squares. The y intercept for the middle and right panels corresponds to a rate of 944 and 973 nmol·g⁻¹·hour⁻¹, respectively.

Together, these various experimental results provide the basis for four general conclusions concerning the movement of cholesterol from circulating lipoproteins of intestinal origin into the liver and suppression of hepatic cholesterol synthesis. First, in all of these studies there is generally a correlation between the clearance of infused lipoprotein cholesterol, elevation of the hepatic cholesterol content, and inhibition of cholesterol synthesis by the liver. Net clearance from the blood of cholesterol carried in intestinal lipoproteins appears to take place only in the liver and is associated exclusively with an increase in the hepatic cholesterol ester fraction. While cholesterol esters can apparently be taken up into the hepatocyte intact from chylomicrons (25, 26), our data do not necessarily imply that only esters move into the cell. Indeed, from what is known about transport of cholesterol across other membranes it is probably the more amphipathic, unesterified cholesterol species that more rapidly penetrates the cell membrane, following which it presumably is esterified for temporary storage in the liver cell.

Second, it is also apparent from these acute studies that the rate of net cholesterol uptake from circulating intestinal lipoproteins is significantly faster than the rate of net disposal of cholesterol esters from the hepatic pool. The apparent velocity of this uptake step can be fairly accurately calculated from those experiments where the interval between the time of injection and killing was only 80 min (Fig. 2). In this study the content of hepatic cholesterol esters increased by 0.015 mg·g⁻¹ for each 1.0 mg·100 g⁻¹ of cholesterol that was injected. Since this increase appears to be a linear function of time, this proportionality constant can be used to calculate a rate constant for uptake of cholesterol by the liver that equals 0.011 (mg·g⁻¹)·(mg·100 g⁻¹)⁻¹ (hour)⁻¹. Using the mean animal and liver weights in this group of rats (200 g and 6.9 g, respectively) this value corresponds to the rate of net cholesterol uptake from circulating intestinal lipoproteins.
The animals in Line 1 were adapted to light cycling for 3.5 weeks and were then fed 18 g of ground rat chow/24 hours for 2 days prior to killing. Different groups of these rats received chow containing 0, 0.1, 0.3, 0.8, and 1.2% added cholesterol. Thus, these different groups had a total intake during this 48-hour period of 0, 36, 108, 288, and 432 mg of cholesterol. This intake was normalized to 100 g of animal weight. The data in Line 2 were derived from the experiments shown in Fig. 3. The data in Lines 3 and 4 were obtained in animals that were continuously infused with intestinal lipoproteins for either 24 or 48 hours as described in Fig. 4. The total dose of cholesterol given in either infusion period was normalized to 100 g of animal weight. Column A gives the total number (n) of animals used to construct each regression curve. Column B gives the mean animal weights and liver weights for all animals in each group. The data in Column G represent the percentage of the total amount of cholesterol administered to the animals accounted for in the hepatic cholesterol ester fraction at the time the animals were killed. These values were calculated by multiplying the values in Column G times the value in Column F times 10^6 and dividing the mean animal weight from Column E. The values in Columns B, C, and D were determined as the slope of the line fitted to the data by the least squares method and include ±1 S.D. The data in Columns B and F are mean values ± 1 S.E.

<table>
<thead>
<tr>
<th>Experimental means of cholesterol administration</th>
<th>A. n</th>
<th>B. Hepatic cholesterol esters/cholesterol input</th>
<th>C. Cholesterol synthesis/cholesterol input</th>
<th>D. In Cholesterol synthesis/hepatic cholesterol esters</th>
<th>E. Mean animal weight</th>
<th>F. Mean liver weight</th>
<th>G. Cholesterol administered in liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cholesterol fed for 48 hrs prior to killing</td>
<td>15</td>
<td>(mg g⁻¹) (mg·100 g⁻¹)⁻¹</td>
<td>-0.018 ± 0.001 (38.3 mg)</td>
<td>-1.04 ± 0.12 (0.66 mg·g⁻¹)</td>
<td>190 ± 5</td>
<td>6.8 ± 0.2</td>
<td>6.1</td>
</tr>
<tr>
<td>2. Bolus of lipoproteins injected 12 hrs prior to killing</td>
<td>29</td>
<td>+0.089 ± 0.005 (8.4 mg)</td>
<td>-0.069 ± 0.009 (0.88 mg·g⁻¹)</td>
<td>-0.78 ± 0.13 (5.5)</td>
<td>195 ± 10</td>
<td>7.2 ± 0.8</td>
<td>32.0</td>
</tr>
<tr>
<td>3. Lipoproteins continuously infused for 24 hrs prior to killing</td>
<td>25</td>
<td>+0.015 ± 0.005 (13.5 mg)</td>
<td>-0.051 ± 0.006 (0.49 mg·g⁻¹)</td>
<td>-1.41 ± 0.38 (3.7)</td>
<td>192 ± 6</td>
<td>7.1 ± 0.3</td>
<td>5.5</td>
</tr>
<tr>
<td>4. Lipoproteins continuously infused for 48 hrs prior to killing</td>
<td>20</td>
<td>+0.010 ± 0.002 (13.5 mg)</td>
<td>-0.051 ± 0.007 (2.0 mg·g⁻¹)</td>
<td>-3.51 ± 0.54 (3.7)</td>
<td>185 ± 4</td>
<td>6.9 ± 0.2</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Table III

Correlation of hepatic cholesterol ester content and rates of cholesterogenesis to cholesterol input

Table III shows the correlation of hepatic cholesterol ester content and rates of cholesterogenesis to cholesterol input and to level of cholesterol esters in liver.

The validity of these two rate constants can be tested using the data from the animals injected with a bolus of lipoproteins 12 hours prior to the time they were killed (Fig. 3, and Table III, Line 2). For example, if a dose of 30 mg·100 g⁻¹ is administered to these animals, the rate constant for the uptake step would predict that at 12 hours the liver should have taken up 26.7 mg of cholesterol, or 46% of the administered dose. During this time the rate constant for degradation would predict the removal of 4.9 mg of cholesterol from the liver so that, theoretically, at the time the animals were killed 37% of the administered dose of cholesterol should be found in the hepatic cholesterol ester fraction. This predicted value corresponds closely to the actual value of 32.9% found in these studies (Table III, Line 2, Column G).

Third, in all of these studies the accumulation of cholesterol esters in the liver appears to be a linear function of the amount of cholesterol injected into the animals even when the dose administered resulted in serum cholesterol levels well above those encountered under physiological circumstances. This is true in the experiments using both the bolus injection and continuous infusion of lipoproteins. Thus, there is no evidence for a saturable step in the uptake of cholesterol from this lipoprotein fraction into the liver cell. This is compatible with other data on the transport of lipids, both sterols and fatty acids, across cell membranes where the translocation step invariably has been found to be passive and so driven by the prevailing electrochemical gradient (27-29).

Fourth, while there is a general correlation between the elevation of hepatic cholesterol ester content and the inhibition of hepatic cholesterogenesis, there is no constant quantitative relationship between the parameters of these two activities. As shown in Fig. 4, for example, a similar degree of inhibition of cholesterogenesis was seen under circumstances where the cholesterol ester content of the liver cell was varied widely by changing the time period over which the infusion was carried out. This same finding is seen in more quantitative terms in the four experimental groups summarized in Table III. As seen in Column B, the cholesterol ester content of the liver increased by 0.017, 0.089, 0.015, and 0.010 mg·g⁻¹, respectively, for each mg·100 g⁻¹ of cholesterol administered by feeding, bolus injection, or continuous infusion for 24 or 48 hours. Similarly, the amount of inhibition of hepatic cholesterogenesis (Column C) expressed per mg·100 g⁻¹ of cholesterol given the animals, varied markedly depending upon the method and time course of administration. The most critical data are shown in Column D where it is evident that cholesterogenesis was inhibited by a factor that varied from 0.78 to 3.51 for an incremental increase in hepatic cholesterol ester content of 1.0 mg·g⁻¹. These data suggest two possibilities with respect to the nature of the inhibitor of hepatic cholesterogenesis.
under these circumstances. If esterified cholesterol is the effector, then only the ester level in some particular subcompartment of the cell, e.g. the nucleus, may act to regulate the level of \( \beta \)-hydroxy-\( \beta \)-methylglutaryl-CoA reductase activity. Alternatively, the effector may be a very small pool of unesterified cholesterol (or some other metabolic product of cholesterol) that cannot be detected by changes in the total cell content of free cholesterol, while cholesterol ester is merely an inert storage form of sterol within the hepatocyte that plays no direct role in inhibiting hepatic cholesterogenesis. Which of these possibilities is correct cannot be determined on the basis of the data available. However, the complexity of the problem is emphasized by these studies in that the level of cholesterol ester in the hepatocyte in any particular experimental setting is a complicated function of the rate of disposition of cholesterol from the liver. In addition, it is also apparent that the potential inhibitory effect of any level of cellular cholesterol or cholesterol esters (or both) must be superposed upon a constantly changing baseline of \( \beta \)-hydroxy-\( \beta \)-hydroxy-\( \beta \)-methylglutaryl-CoA reductase due to the normal diurnal variation in activity of this enzyme. Thus, it is not surprising that there is no direct quantitative relationship between the level of \( \beta \)-hydroxy-\( \beta \)-methylglutaryl-CoA reductase and the rate of cholesterol synthesis.

Acknowledgments—The authors wish to acknowledge the excellent technical assistance of Joyce Eckles, Dorothy Lu, and Claudette Keel.

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