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

(Received for publication, June 2, 1975)  

FLAVIO O. NERVI and JOHN M. DIETCHFY  
From the Gastrointestinal-Liver Section of the Department of Internal Medicine, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235

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 \( \beta \)-hydroxy-\( \beta \)-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 light 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

*This work was supported by United States Public Health Service Research Grants HL 09610 and AM 16386 and by United States Public Health Service Institutional Research Training Grant GM 00034.

‡ Partially supported by a grant from Catholic University of Chile during the period when these studies were being carried out. Present address, Departamento de Gastroenterología, Universidad Católica de Chile, Casilla 114-D, Santiago, Chile.
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 Dissé, 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 4 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 removed under anesthesia by means of aortic puncture and was fitted with an indwelling gastric and intravenous catheter. The animal was killed and liver slices were prepared. In a second group of studies donor rats were fed ground Formulab chow diet that was not supplemented with either corn oil or cholesterol. The serum obtained having mean densities equal to <1.006, 1.006 to 1.070, and 1.070 to 1.215 were dialyzed against three changes (4 liters) of 0.9% NaCl solution at 4°C for 12 hours. The total cholesterol content of each fraction was then determined and the concentrations of the lipoprotein solutions were adjusted to the desired value using 0.9% NaCl solution. In all experiments the amount of a particular lipoprotein administered to an assay animal is expressed as the milligrams of total cholesterol contained in that fraction and normalized to 100 g of animal weight, i.e., mg 100 g⁻¹.

**Fractionation of all intestinal lymph samples was carried out in SW 25.1 swinging bucket rotors while serum fractions were obtained using 60 Ti fixed angle titanium rotors. The data in Column D represent the total lipid extracted with chloroform/methanol (2/1) present in each lipoprotein fraction per mg of total cholesterol: mean values ± 1 S.E. for 7 to 18 samples are given.**

| A. Designation of fraction | B. Source of lipoproteins | C. Conditions of centrifugation for isolation | D. Total lipids (mg) (mg total cholesterol) | mg 100 g⁻¹ |
|---------------------------|--------------------------|-------------------------------------------|------------------------------------------|-----------|
| S, > 8000                 | Intestinal lymph         | 16,000 rpm x 10 min                       | 138 ± 7                                  |           |
| S, 400-8000               | Intestinal lymph         | 22,000 rpm x 30 min                       | 88 ± 6                                   |           |
| S, 30-400                 | Intestinal lymph         | 22,000 rpm x 24 hr                        | 35 ± 3                                   |           |
| d < 1.006                 | Serum                    | 50,000 rpm x 24 hr                        | 17 ± 2                                   |           |
| d 1.006-1.070             | Serum                    | 60,000 rpm x 24 hr                        | 8 ± 1                                    |           |
| d 1.070-1.215             | Serum                    | 60,000 rpm x 24 hr                        | 5 ± 2                                    |           |

**Whole serum**

**Lipoproteins** — Serum 60,000 rpm x 24 hr 6 ± 2

**The abbreviation used is:** HMG-CoA reductase, β-hydroxy-β-methylglutaryl-CoA reductase.
All assay animals used in this study were subjected to light cycling for 2.5 weeks prior to use. Each animal was then removed from the cycling chamber at the mid-light phase of the cycle and injected with a bolus of one of the four different lipoprotein fractions containing varying amounts of total cholesterol. The assay animals were returned immediately to the light cycling chamber and were killed 12 hours later at the mid-dark phase of the light cycle. Various metabolic pathways were then measured in the liver as shown in Fig. 2. Column A gives the total number (n) of animals used to construct each regression curve. Column B gives the incremental change in cholesterol ester content of the liver for each milligram 100 g⁻¹ of cholesterol injected into the assay animal. Columns C and D relate the natural logarithm (ln) of the rates of hepatic cholesterogenesis to the amount of cholesterol administered in each lipoprotein fraction and to the level of hepatic cholesterol esters, respectively. The relationships shown in Columns C and D are given both as the first order constants for the semi-logarithmic plots (k) and as the amount of cholesterol administered or the increment in hepatic cholesterol ester content necessary to achieve 50% inhibition of the rate of cholesterogenesis by the liver (numbers in parentheses). Columns E and F, respectively, give 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 that is 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 B times the value in Column F times 10⁴ and dividing by 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 (as seen in Fig. 2) and include ± 1 S.D.; the data in Columns E and F are mean values ± 1 S.E.

**Table II**

| Lipoprotein fraction administered | A. n | B. Hepatic cholesterol esters/cholesterol input | C. In cholesterol synthesis/cholesterol input | D. In cholesterol synthesis/hepatic cholesterol esters | E. Mean animal weight | F. Mean liver weight | G. Cholesterol administered in liver |
|----------------------------------|------|-----------------------------------------------|-----------------------------------------------|--------------------------------------------------|----------------------|---------------------|-----------------------------------|
|                                  |      | (mg g⁻¹)                                      | (mg 100 g⁻¹)                                  |                                                  |                      |                     |                                   |
|                                  |      | (mg 100 g⁻¹)                                  |                                               |                                                  |                      |                     |                                   |
| S₄ > 8000                        | 40   | +0.108 ± 0.011                                | -0.106 ± 0.025                                | -1.02 ± 0.17                                     | 205 ± 6              | 7.5 ± 0.1           | 39.5                              |
| S₄ 400–8000                      | 23   | +0.071 ± 0.012                                | -0.097 ± 0.011                                | -1.31 ± 0.16                                     | 192 ± 7              | 7.2 ± 0.2           | 26.6                              |
| S₄ 30–400                        | 36   | +0.031 ± 0.009                                | 0.112 ± 0.027                                 | 1.78 ± 0.14                                     | 106 ± 10             | 6.0 ± 0.3           | 10.9                              |
| Whole serum lipoproteins         | 14   | +0.002 ± 0.003                                | -0.035 ± 0.015                                | -0.83 ± 0.51                                     | 201 ± 4              | 7.0 ± 0.6           | 0.7                               |

The incorporation rates of [1-¹⁴C]octanoate into cholesterol and CO₂ 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 (16, 17). Rates of cholesterogenesis or CO₂ production were calculated as the nanomoles and micromoles, respectively, of acetyl-CoA, i.e. C₅ units, incorporated into cholesterol and CO₂ per gram wet weight of liver slices per hour of incubation, i.e. nanomoles g⁻¹ hour⁻¹ and micromoles g⁻¹ hour⁻¹. These rates of C₅ flux into cholesterol mirror exactly the rates of HMG-CoA reductase activity in the same livers (17). Aliquots of blood, liver, and the lipoprotein fraction injected were also obtained for determination of total cholesterol and cholesterol ester content (18, 19).

**Mathematical Treatment of Data**—Where appropriate, mean values for groups of data are given ± 1 S.E. For correlating two variables, 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 when x equals zero 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 (2) and for lipoprotein injection (4, 15), the rate of hepatic cholesterogenesis appears to vary in a log-linear fashion with the amount of cholesterol administered to the animal or with the hepatic cholesterol ester content. Hence, in studies such as those shown in Fig. 2, the natural logarithm, ln, of the C₅ flux into cholesterol was used for regression analysis of the relationship between the rate of cholesterol synthesis and the amount of cholesterol administered in a particular lipoprotein fraction or the level of hepatic cholesterol ester content.

**RESULTS AND DISCUSSION**

Previous work published from this laboratory has characterized the general features of the control of hepatic cholesterogenesis 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 cholesterogenesis. 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 cholesterogenesis 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 C₅ 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. 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 base-line cholesterogenic activity in the two types of assay procedures utilized in these studies is also shown at the
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 $S_r > 8000$ fraction. These alterations in cholesterol ester content and rate of cholesterol synthesis occurred under circumstances where the rate of hepatic ketone synthesis ($F$) and $C_2$ flux into CO$_2$ ($E$) 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 $S_r$ values of 400 to 8000 and 30 to 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 $S_r > 8000$ (0.071 mg g$^{-1}$), $S_r$ 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 $S_r > 8000$ 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 cholesterogenesis (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 cholesterogenesis 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 cholesterogenesis 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 increment 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 $S_2 > 8000$ 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), CO$_2$ production (E), and ketone synthesis (F), and serum cholesterol levels (D) are plotted as a function of the amount of cholesterol injected 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.

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 $S_2 > 8000$ and $S_2 30$ to 400 fractions but at both 12 and 24 hours the ester content had declined much more in those animals injected with the $S_2 30$ 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 continuous intravenous infusion for a 24-hour period. The same three proteins of intestinal origin.

protein fractions under the more physiological circumstance where they were administered in low concentrations by continuous 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 cholesterogenesis synthesis. As shown in Column B, the observed increase in cholesterol esters was greatest with the S₃ > 8000 fraction (0.053) and decreased as the intestinal lipoprotein fractions with S₃ 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 S₃ > 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 S₃ > 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 III). As seen in Column C, however, the fractional rate of inhibition was nearly 6-fold greater when the S₃ > 8000 intestinal lipoproteins were infused than when the S₃ 30 to 400 fractions were administered. However, since the incremental increase in cholesterol esters was disproportionately lower (Column B) for the S₃ 400 to 8000 and S₃ 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 (S₃ > 8000) to 4.21 (S₃ 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 30 times that seen with the S₃ > 8000 intestinal lipoproteins, and, as with the bolus injections, there was no significant

![Diagram](http://www.jbc.org/)

Fig. 3. Detailed time courses for the clearance of serum cholesterol and the change in hepatic cholesterol ester levels after the administration of a bolus of either S₃ > 8000 or S₃ 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.
difference among these three serum lipoprotein fractions.

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 3S 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 injected with the 3S > 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 (3, 26, 27).

A third major conclusion that is supported by these studies is that the serum cholesterol ester content and rates of cholesterol synthesis are not a significant factor in regulating the rate of cholesterogenesis.

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

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.

| Lipoprotein fraction administered | A. hepatic cholesterol esters/cholesterol input | B. in cholesterol synthesis/cholesterol input | C. in cholesterol synthesis/hepatic cholesterol esters | D. cholesterol administered in liver | E. mean animal weight | F. mean liver weight | G. cholesterol synthesis/hepatic cholesterol esters |
|----------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|----------------------------------|----------------------|----------------------|---------------------------------------------|
| S < 8000                         | 9 +0.055 ± 0.014                           | -0.084 ± 0.015                              | -1.17 ± 0.31                               | 192 ± 6                         | 7.2 ± 0.2            | 19.9 |
| S 400-800                        | 10 +0.014 ± 0.004                           | -0.040 ± 0.018                              | -1.95 ± 0.80                               | 201 ± 5                         | 6.9 ± 0.1            | 4.8 |
| S 30-400                         | 10 +0.004 ± 0.002                           | -0.013 ± 0.005                              | -4.21 ± 1.01                               | 196 ± 2                         | 7.2 ± 0.3            | 1.5 |
| d < 1.006                        | 13 +0.003 ± 0.003                           | -0.021 ± 0.015                              | -1.20 ± 0.15                               | 204 ± 7                         | 7.4 ± 0.2            | 1.1 |
| d 1.006-1.700                    | 11 +0.009 ± 0.005                           | -0.012 ± 0.006                              | -0.73 ± 0.25                               | 189 ± 5                         | 6.7 ± 0.3            | 3.2 |
| d 1.700-215                      | 9 +0.014 ± 0.010                            | -0.009 ± 0.015                              | -0.77 ± 0.12                               | 194 ± 3                         | 6.8 ± 0.2            | 1.9 |
that the amount of inhibition of hepatic cholesterogenesis 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 effectors 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 cholesterol load to lipoprotein, respectively, and can be compared to a rate of uptake of 3.8% of the cholesterol load administered as unfractonated, 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 monomer 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 S, 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 cholesterogenesis is directly related to the S, value of the fraction of intestinal lipoproteins injected (Table III).

Acknowledgments—We wish to acknowledge the excellent technical assistance of Joyce Ekedee, Dorothy Lu, and Claudtte Keel.

REFERENCES
1. Weis, H. J., and Dietschy, J. M. (1975) Biochim. Biophys. Acta 398, 215–224
2. Gould, R. G., and Swoyry, E. A. (1966) J. Lipid Res. 7, 688–707
3. Harry, D. S., Dini, M., and McEntyre, N. (1973) Biochim. Biophys. Acta 296, 280–290
4. Nervi, F. O., Weis, H. J., and Dietschy, J. M. (1975) J. Biol. Chem. 250, 4145–4151
5. Sakakida, H., Sheidacs, C. C., and Siperstein, M. D. (1963) J. Clin. Invest. 42, 1521–1528
6. Siperstein, M. D. (1960) Am. J. Clin. Nutr. 8, 645–650
7. dietschy, J. M., and Westergaard, H. (1975) in Intestinal Absorption and Malabsorption (Csaky, T. Z., ed), pp. 191–207, Raven Press, New York
8. Sallee, V. L., and Dietschy, J. M. (1973) J. Lipid Res. 14, 475–484
9. Westergaard, H., and Dietschy, J. M. (1974) J. Clin. Invest. 54, 718–732
10. Sherrill, B. C., and Dietschy, J. M. (1975) J. Membrane Biol., in press
11. Kandutsch, A. A., and Saurier, S. F. (1969) J. Biol. Chem. 244, 2299–2305
12. Hampsrech, B., Nissler, C., and Lynene, F. (1969) FEBS Lett. 4, 117–121
13. Doke, V. P., and Hamlin, J. T. (1962) Physiol. Rev. 42, 674–701
14. Hatch, F. T. (1969) Adv. Lipid Res. 6, 1–68
15. Weis, H. J., and Dietschy, J. M. (1969) J. Clin. Invest. 48, 2398–2408
16. Dietschy, J. M., and McGarry, J. D. (1974) J. Biol. Chem. 249, 53–68
17. Dietschy, J. M., and Brown, M. S. (1974) J. Lipid Res. 15, 508–516
18. Wilson, J. D. (1963) Circ. Res. 12, 472–478
19. Nervi, F. O., and Dietschy, J. M. (1974) Biochim. Biophys. Acta 369, 351–360
20. Higgins, M., and Rudney, H. (1973) Nature New Biol. 246, 80–81
21. Edwards, P. A., and Gould, R. G. (1972) J. Biol. Chem. 247, 1520–1524
22. Doke, V. P., and Dietschy, J. M. (1974) J. Biol. Chem. 249, 147–155
23. Weis, H. J., and Dietschy, J. M. (1973) Gastroenterology 64, 77–94
24. Cooper, A. D., and Ockner, R. K. (1974) Gastroenterology 66, 586–595
25. Seidel, D., Alaupovic, P., Furman, R. H., and McConathy, W. J. (1970) J. Clin. Invest. 49, 2386–2407
26. Redgrave, T. G. (1970) J. Clin. Invest. 49, 465–471
Ability of six different lipoprotein fractions to regulate the rate of hepatic cholesterogenesis in vivo.
F O Nervi and J M Dietschy

J. Biol. Chem. 1975, 250:8704-8711.

Access the most updated version of this article at http://www.jbc.org/content/250/22/8704

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/250/22/8704.full.html#ref-list-1