Tissue distribution of cholesterol feedback control in the guinea pig

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Abstract  The level of cholesterol synthesis and the activity of the cholesterol feedback system were studied in tissue slices from a number of organs of the guinea pig. In contrast to the tissue distribution of sterol synthesis in the rat, liver slices of the guinea pig have a low rate of sterologenesis, with ileum and lung being the most active sterologenic tissues. More surprising, all tissues studied in the guinea pig, including lung, ileum, and brain, were shown to possess an active cholesterol feedback system. The basis for the widespread organ distribution of cholesterol feedback control in the guinea pig is probably the ability of the various tissues of the guinea pig to take up and concentrate exogenous cholesterol and is not the result of any inherent differences in the lipoprotein composition in this species.

Supplementary key words  lung · synthesis · spleen · intestine · Triton · brain · liver

While it has been known for over two decades that under in vitro conditions every mammalian tissue examined is capable of synthesizing cholesterol from acetate (1), the liver is generally believed to be the chief source of endogenous cholesterol in higher animals. Consistent with this major role in sterol synthesis, it is well established that in all species studied (2-7), including man (8), cholesterogenesis in the liver is subject to a sensitive negative feedback control system by which exogenous cholesterol depresses hepatic cholesterol production through a specific inhibition of the synthesis of mevalonic acid (9-11). Extensive studies have also lent strong support to the current consensus that this cholesterol-sensitive cholesterol feedback system is limited only to liver (12).

It should be emphasized, however, that the latter conclusion is based upon studies carried out primarily in the rat, with one report demonstrating a similar pattern in the squirrel monkey (13) and one other (4) demonstrating that in the dog this feedback response is also restricted only to liver. To determine whether this limited tissue distribution of cholesterol feedback control applies to other species, we examined in vitro the tissue localization of both cholesterol synthesis and the cholesterol feedback system in another widely used laboratory animal, the guinea pig.

The results demonstrate, first, that in this species hepatic sterol synthesis proceeds only slowly, with the lung and intestine of the guinea pig representing the most active sites of sterologenesis. Second and most unexpectedly, in contrast to the restricted hepatic localization of cholesterol feedback control observed in the rat (12), monkey (13), and probably the dog (4), the cholesterol feedback system in the adult guinea pig is present in all tissues studied, including lung, spleen, adrenal, brain, intestine, and lymph node.

MATERIALS AND METHODS

Animals and diets

Female guinea pigs weighing 300-500 g were used in this study. Control and Triton-treated animals were fed ad lib. Purina guinea pig chow to which was added oleic acid to a total concentration of 10%. This diet contained less than 2 mg of cholesterol per gram, as determined by gas-liquid chromatography. Cholesterol-fed animals were given Purina lab chow containing 10% oleic acid and 5% cholesterol for 7 days prior to the in vitro studies.

Triton-treated animals were injected intraperitoneally with 250 mg of Triton WR-1339 (oxyethylated t-octyl phenol, obtained from Winthrop-Stearns, Inc., New York) in 2.5 ml of normal saline on three successive evenings and were killed 18 hr after the third injection.

Tissue preparation

The animals were exsanguinated between 9:00 and 10:00 a.m.; their organs were promptly removed, washed...
with cold saline, and placed in beakers of Krebs-Ringer phosphate buffer (pH 7.4) at 0°C. Tissue slices 1 mm thick were prepared with a McIlwain tissue slicer (M. Mickel, Gomshall, Surrey, England). 200 mg of each tissue (except adrenal, of which 100 mg was used) was placed in a 25-ml center-well flask containing 2 ml of Krebs bicarbonate buffer, 2 μCi of sodium [2-14C]acetate (sp act 2 μCi/μmole; New England Nuclear Corp., Boston, Mass.), and 5 μmoles of unlabeled sodium acetate. The flasks were gassed with 95% O2–5% CO2 and stoppered, and the samples were incubated at 37°C for 2 hr in a Dubnoff metabolic shaker at 100 oscillations/min.

Isolation of 14C-labeled sterols, fatty acids, and CO2

The analytical procedures were essentially those previously described from this laboratory (9). After incubation, the contents of the flasks were acidified with 1 N H2SO4, and 0.3 ml of 1 M Hyamine solution (Packard Instrument Co., Downers Grove, Ill.) in methanol was added to the center wells. The flasks were shaken at room temperature for 45 min, and an aliquot of the Hyamine solution was assayed for 14CO2 in a 2,5-diphenyloxazole-1,4-bis[2-(5-phenyloxazolyl)]-benzene (PPO-POPOP) mixture using a Beckman liquid scintillation counter.

The samples were then alkalized and saponified, and the alkaline hydrolyzates were extracted three times with 5 vol of petroleum ether. 3-β-Hydroxysteroids were precipitated with digitonin and dried, and an aliquot was assayed for 14C as previously described (5). It is, of course, recognized that other sterols besides cholesterol may be included in this digitonin-precipitable fraction, and the term “sterol synthesis” will therefore be employed to designate the 14C incorporated into digitonin-precipitable sterols.

Total tissue cholesterol

Duplicate aliquots of digitonin-precipitable sterol were dried and dissolved in 2 ml of acetic acid. 4 ml of acetic anhydride–sulfuric acid 20:1 was added, and after 35 min the resulting color was read at 660 nm on a Gilford spectrophotometer. Cholesterol-containing lipoproteins were prepared after feeding roosters Purina Layena chicken mash, to which was added 5% cholesterol and 10% corn oil, and bleeding them from the wing veins after 1–3 wk on this diet. The cholesterol-rich lipoproteins were isolated from the serum by centrifugation at 100,000 g for 16 hr. The infranate was then removed, and the uppermost layer containing the lipoproteins was washed twice by thorough mixing with 0.9% saline and recentrifugation at 100,000 g for 2 hr. The resulting washed lipoproteins were resuspended in 0.9% saline prior to injection. The cholesterol concentration of this fraction was 1060 mg/100 ml, and the triglyceride concentration was 249 mg/100 ml.


d It is recognized that this preparation of chicken lipoproteins may contain chylomicron remnants and very low density lipoproteins as well as chylomicrons.

### RESULTS

Sterol synthesis: its feedback control and response to Triton in various tissues of the guinea pig

As shown in Table 1, under the in vitro conditions employed, liver slices from normal guinea pigs fed a low cholesterol diet synthesize sterols at a relatively slow rate, which averages only 4% of that of rat liver and is only 6% of that of the guinea pig ileum. On the other hand, all of the other tissues examined in the guinea pig were found to synthesize sterols at rates significantly greater than the corresponding tissue of the rat. Triton administration (Table 2), however, increased hepatic sterol synthesis in the guinea pig 17-fold, demonstrating that the guinea pig liver has the potential for very rapid sterol production. As also indicated in Table 2, feedback control of sterol synthesis in the guinea pig liver is very effective; in these experiments cholesterol feeding for 1 wk reduced hepatic sterol synthesis to 6% of that of the normal liver and to 0.4% of that of the Triton-treated liver. These results therefore demonstrate that, in the guinea pig, hepatic sterol synthesis is under sensitive feedback control and that, moreover, even in the normal animal fed a relatively low cholesterol diet, sterologenesis is greatly suppressed, presumably by the feedback effect of endogenous cholesterol.

### TABLE 1. Sterol synthesis in seven tissues of rat and guinea pig

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Rate of Acetate Incorporation into Sterol (nmol/g/2 hr ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (9)b</td>
<td>179.0 ± 48.7</td>
</tr>
<tr>
<td>Ileum (7)</td>
<td>114.3 ± 27.5</td>
</tr>
<tr>
<td>Lung (9)</td>
<td>6.7 ± 0.1</td>
</tr>
<tr>
<td>Adrenal (7)</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>Spleen (7)</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>Lymph node (6)</td>
<td>10.5 ± 2.5</td>
</tr>
<tr>
<td>Brain (6)</td>
<td>0.5 ± 0.2</td>
</tr>
</tbody>
</table>

- After Dietschy and Siperstein (12).
- Numbers in parentheses indicate the number of guinea pigs studied.

### TABLE 2. Effect of cholesterol feeding and Triton treatment on sterol synthesis in seven guinea pig tissues

<table>
<thead>
<tr>
<th>Sterol Synthesis</th>
<th>Normal</th>
<th>5% Cholesterol</th>
<th>Triton</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (9)</td>
<td>7.9 ± 1.4</td>
<td>0.5 ± 0.1</td>
<td>132.0 ± 23.3</td>
</tr>
<tr>
<td>Ileum (7)</td>
<td>130.0 ± 13.4</td>
<td>37.0 ± 1.5</td>
<td>357.5 ± 35.1</td>
</tr>
<tr>
<td>Lung (7)</td>
<td>22.2 ± 2.8</td>
<td>4.9 ± 0.8</td>
<td>81.5 ± 10.5</td>
</tr>
<tr>
<td>Adrenal (6)</td>
<td>13.1 ± 1.7</td>
<td>3.8 ± 0.8</td>
<td>19.6 ± 4.0</td>
</tr>
<tr>
<td>Spleen (9)</td>
<td>10.5 ± 2.6</td>
<td>0.5 ± 0.2</td>
<td>16.1 ± 4.1</td>
</tr>
<tr>
<td>Lymph node (7)</td>
<td>6.5 ± 0.6</td>
<td>1.3 ± 0.3</td>
<td>6.0 ± 0.6</td>
</tr>
<tr>
<td>Brain (6)</td>
<td>4.9 ± 0.5</td>
<td>0.9 ± 0.3</td>
<td>4.1 ± 0.6</td>
</tr>
</tbody>
</table>

- Numbers in parentheses indicate the number of animals studied.
Ileum

The ileum was by far the most active sterol-producing organ studied in the normal guinea pig. It is of interest that the major sterol synthesized by the guinea pig intestine, as shown by Ockner and Laster (14), is not cholesterol but lathosterol. Triton administration resulted in a maximal rate of sterol synthesis that was also the most rapid of any tissue examined in this species, being nearly three times that of liver.

Sterologenesis in the ileum is also very sensitive to dietary cholesterol but not so much so as is liver; i.e., sterol synthesis in the ileum was feedback-suppressed to 28% of normal while the comparable inhibition in the liver amounted to 6% of the control value. As a result, in the cholesterol-fed as well as in the normal guinea pig, the intestine represents the major site of sterol synthesis in vitro.

Lung

The results obtained in the lung provided the most unexpected findings of this study. On an average, lung slices of the normal guinea pig produce sterol at a rate three times that of liver, a rate more rapid than that in any other organ studied except the ileum. Triton administration increases sterol synthesis in lung fourfold to a maximal rate approaching that of the Triton-stimulated liver. On the other hand, dietary cholesterol markedly suppresses sterol synthesis in the lung to a rate 22% of that of the animal on a low cholesterol diet. Thus, guinea pig lung is capable of very active sterol synthesis and, most unexpectedly, pulmonary tissue possesses a very sensitive cholesterol feedback system.

Adrenal

Adrenal is also capable of synthesizing sterols at a significant rate; per weight of tissue the rate is nearly twice that of the liver in the normal animal. Triton administration elicits only a modest increase in sterologenesis in this tissue; however, dietary cholesterol was found to suppress sterol synthesis in the adrenal by at least 70%.

Spleen

In the guinea pig, spleen normally synthesizes sterols at a rate per unit weight that is very similar to that of liver. Sterologenesis in the spleen is, moreover, significantly suppressed by dietary cholesterol; however, the results of Triton treatment indicate that, unlike the liver, the spleen in the normal animal synthesizes sterols at about 70% of its maximal rate, i.e., it is not significantly stimulated by the Triton administration.

Lymph node

Lymph node synthesized sterols less rapidly than did spleen and was also less sensitive to control by exogenous sterol. On the basis of the results of Triton stimulation, it appears that the lymph node in the normal animal produces sterols at or near its maximal rate.

Brain

Unexpectedly, brain slices of the adult guinea pig are capable of synthesizing sterols at a rate per gram approaching that of liver. Moreover, sterol synthesis in the guinea pig brain is depressed by dietary cholesterol to 20% of normal. The failure of Triton treatment to stimulate sterol synthesis suggests that, in the adult guinea pig on a low cholesterol diet, the brain synthesizes sterol at or near its maximal rate.

Cholesterol content of guinea pig tissues after cholesterol feeding

The effects of cholesterol feeding on the tissue concentrations of cholesterol are summarized in Table 3. The cholesterol-fed guinea pig accumulated cholesterol in every organ studied; however, in terms of total cholesterol per unit weight of tissue, liver and adrenal concentrated the greatest amounts of cholesterol. The lung, followed by spleen and lymph node, showed lower levels of cholesterol; ileum accumulated very little cholesterol. When the level of this cholesterol accumulation is compared with the normal cholesterol content of these various tissues, it is found that the liver becomes the chief depot of exogenous cholesterol, more than tripling its cholesterol content. It is, however, apparent from the data in Tables 2 and 3 that the absolute tissue concentrations of cholesterol in guinea pigs fed either a high or a low cholesterol diet are poorly correlated with the relative rates of cholesterol synthesis in the various organs studied.

Sterol synthesis from [1-14C]octanoate

Because dilution of acetate or differences in the activation of acetate to acetyl CoA might conceivably account for some of the differences in conversion of acetate to sterols in the guinea pig tissues, sterol synthesis was quantified in a
single experiment in which [1-14C]octanoate was employed as the source of the labeled active acetate (acetyl CoA). Although it is recognized that differential dilution of substrate from one tissue to another might also occur with octanoate, demonstration of sterol synthesis and its feedback control with a second substrate would strengthen the conclusion that this system has a widespread tissue distribution in the guinea pig.

The results of this experiment (Table 4) demonstrate that, when employed as sterol precursors in guinea pig tissues, acetate and octanoate yield very similar results. Both the level of sterol synthesis and the degree of feedback inhibition in the three tissues studied, i.e., liver, lung, and spleen, were approximately the same with the two substrates. It should be noted that in this one experiment the incorporation of both [14C]acetate and [14C]octanoate into sterols by the liver was higher than in previous experiments; however, even in this experiment hepatic sterol synthesis by the guinea pig was far less than that seen in the rat liver.

Effect of intravenous infusion of cholesterol on synthesis and uptake of sterol in guinea pig tissue

The effect of intravenously administered cholesterol-containing lipoproteins on sterol synthesis in guinea pig liver, spleen, and lung was next studied. The injection of hyperlipemic serum from cholesterol-fed chickens has previously been shown (15) to be an effective means of suppressing sterol synthesis not only in guinea pig liver but also in lung and spleen. By contrast, in the rat a similar dose of lipoprotein-bound cholesterol causes suppression of sterol synthesis only in liver, with lung and spleen showing no changes in the rates of sterologenesis. The absolute values for the untreated animals in this study differ somewhat from those in the experiment shown in Table 1; however, this variation is that usually seen in experiments dealing with cholesterol synthesis in isolated tissues. Despite this biological variation, these results again dramatically demonstrate the marked difference between rat and guinea pig tissues. As shown in Table 5, the sensitivity of guinea pig lung and spleen to feedback control by such infused cholesterol is correlated with the uptake of sterol by these tissues. In the rat, in contrast to the guinea pig, neither lung nor spleen accumulates significant quantities of infused cholesterol.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Treatment</th>
<th>Sterol Synthesis</th>
<th>Sterol Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td>Lung</td>
</tr>
<tr>
<td>Rat (4)</td>
<td>Control</td>
<td>322.2 ± 92.6</td>
<td>6.2 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Cholesterol infusion</td>
<td>109.5 ± 3.8</td>
<td>8.1 ± 1.2</td>
</tr>
<tr>
<td>Guinea pig (3)</td>
<td>Control</td>
<td>3.8 ± 0.3</td>
<td>34.9 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Cholesterol infusion</td>
<td>0.4 ± 0.1</td>
<td>20.5 ± 0.3</td>
</tr>
</tbody>
</table>

a14C incorporation from [1-14C]octanoate was multiplied by 4 to correct for incorporation of active C units into sterols.

bExpressed as mg digitonin-precipitable sterol/g tissue.

cNumbers in parentheses indicate the number of animals studied.
The most striking observation in the present study, however, is that every tissue examined in the guinea pig is subject to cholesterol feedback control; the feeding of cholesterol caused an inhibition of sterol synthesis not only in liver (83%) but also in intestine (73%), spleen (95%), adrenal (72%), and brain (80%). In this regard, the results in the lung are of particular note in that, except for the ileum, pulmonary tissue was the most active sterologenic tissue studied, and unexpectedly, lung, too, showed a marked feedback response (78%) to dietary cholesterol. Assuming an average weight of guinea pig lung of 4 g and a liver weight of 15 g, the total sterol production by the lung may be comparable to that of the liver.

Whereas it is well known that the brain of the newborn rodent will synthesize sterols (1), in the rat this process decreases rapidly after birth, and in the adult rat the brain is not capable of significant rates of sterologenesis (1, 12). Clearly, this is not true of the guinea pig in that, even in the 300–500 g adult animal, sterol synthesis in brain slices proceeds at rates that are not significantly less than those of liver.

These observations obviously raise questions, which cannot at present be answered, as to the function of the sterologenic capacity of the various guinea pig tissues that might normally be partially suppressed by endogenous cholesterol. Because this Triton-induced increase in sterol synthesis has been shown to result from a stimulation of hydroxymethylglutaryl CoA reductase (18), the procedure is physiologically meaningful as a means of assessing maximal sterologenic capacity. The results of these studies suggest that the guinea pig liver normally produces sterols at a rate far below its maximal capacity; however, it is apparent from the data in Table 1 that lung and ileum are normally under a significant degree of endogenous feedback control. By contrast, sterologenesis in spleen and adrenal are only slightly stimulated by Triton, and sterol synthesis in brain and lymph nodes is totally unaffected by this treatment. It is likely, therefore, that in the normal guinea pig these tissues are carrying out sterol synthesis at or near their maximal capacities. The results of Triton administration therefore serve to confirm the conclusion that many tissues of the guinea pig are capable of both rapid sterol synthesis and feedback regulation.

In addition to their susceptibility to cholesterol feedback control, each of the nonhepatic tissues of the guinea pig accumulates cholesterol when the animal is fed a high cholesterol diet. An attempt was therefore made to determine whether the sensitivity of the cholesterol feedback system of the various guinea pig tissues to exogenous cholesterol might be related to this ability to accumulate exogenous sterol. The results indicate that, in animals fed either the low or the high cholesterol diet, the total content of cholesterol present in each organ is poorly correlated with the rate of cholesterol synthesis by that tissue. However, as shown in Fig. 1, if the relative amount of cholesterol accumulated, i.e., the cholesterol concentration of the tissue on the high cholesterol diet, divided by its normal concentration, is plotted against the degree of feedback suppression, there is generally a good correlation between the level of such excess cholesterol and the suppression of sterologenesis. The most obvious exception to this relationship occurs in the liver, in which sterol synthesis seems to be far less sensitive to cholesterol accumulation than is the case for other tissues. It is of course possible that this relationship may simply reflect the ability of the liver to accumulate relatively large amounts of cholesterol after endogenous sterol synthesis has been almost completely suppressed. In this regard, however, it should also be noted that Triton stimulation of sterologenesis was not accompanied by a comparable general depletion of tissue sterol. The result is consistent with our earlier suggestion that only specific lipoprotein forms of cholesterol are capable of mediating the cholesterol feedback system (15). Moreover, as we have also previously emphasized, even after cholesterol feeding marked suppression of sterologenesis can occur in liver.
prior to any detectable accumulation of cholesterol (9).

The striking difference in feedback sensitivity between the extrahepatic tissues of rat and guinea pig could presumably result from either of two differences in cholesterol metabolism in these two species. First, cholesterol might be absorbed in the guinea pig in a lipoprotein form that is more readily taken up by various nonhepatic cells than are the cholesterol-containing lipoproteins of the rat. Secondly, the responses of the peripheral tissues of the guinea pig may themselves differ from those of the rat in, for example, the ability of their cells to clear cholesterol-containing lipoproteins from the blood or in the sensitivity of the response of \( \beta \)-hydroxy-\( \beta \)-methylglutaryl coenzyme A reductase, to exogenous cholesterol. To differentiate between these two possible mechanisms, washed chicken lipoproteins containing sufficient cholesterol to cause significant suppression of hepatic cholesterol synthesis (15) were infused into rats and guinea pigs. The results indicate that the feedback sensitivity of the extrahepatic tissues of the guinea pig is not due to any unique property of guinea pig lipoproteins, in that cholesterol-rich lipoproteins obtained from chickens were found to cause definite suppression of sterologenesis in guinea pig liver, lung, and spleen. By contrast, in the rat, comparable infusions of chicken lipoproteins cause suppression of sterol synthesis only in the liver. A corresponding pattern of net cholesterol uptake occurred in these two species. In the guinea pig there was accumulation of cholesterol in liver, spleen, and lung, but only liver showed this response in the rat.

It is reasonable to conclude, therefore, that the widespread feedback sensitivity of sterol synthesis in the extrahepatic tissues of the guinea pig is the result of the general ability of these tissues in the guinea pig to accumulate exogenous cholesterol. Whether this property in turn is due to increased permeability of the guinea pig cell membrane to cholesterol-containing lipoproteins, is the result of an enhanced intracellular cholesterol binding, or is caused by other differences in the ability of guinea pig cells to metabolize cholesterol is currently under investigation.

In summary, these data demonstrate that a wide variety of tissues in the guinea pig are capable of active sterologenesis, a process that in this species is under feedback control by exogenous cholesterol in all tissues studied. It remains to be determined whether other animal species follow the pattern of restricted hepatic feedback control previously demonstrated in rat and monkey or whether, alternatively, other species show the widespread tissue distribution of the cholesterol feedback system demonstrated by this study to be characteristic of the guinea pig.

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