Relative Importance of High and Low Density Lipoproteins in the Regulation of Cholesterol Synthesis in the Adrenal Gland, Ovary, and Testis of the Rat*

(Received for publication, March 17, 1978, and in revised form, July 7, 1978)

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The abbreviations used are: APP, 4-aminopyrazolo[3,4-d]pyrimidine; APP', 4-amino-2-pyrazolopyrimidine; HMG, 3-hydroxy-3-methylglutaryl coenzyme A reductase; ACTH, adrenocorticotropin; hCG, human chorionic gonadotropin; 4-AP, 4-aminopyrazolo[3,4-d]pyrimidine; DPS, digitonin-precipitable sterols; HMG, 3-hydroxy-3-methylglutaryl coenzyme A reductase; ACTH, adrenocorticotropin; hCG, human chorionic gonadotropin.

These studies were undertaken to determine the relative importance of plasma lipoproteins and trophic hormones as regulators of cholesterol synthesis in three endocrine glands of the rat. In rats treated with 4-aminopyrazolo[3,4-d]pyrimidine, the plasma cholesterol level was reduced from 56 to 5 mg/dl and this resulted in a decrease in cholesterol ester content in the adrenal gland from 26.2 to 1.12 mg/g and in the ovary from 4.61 to 2.54 mg/g but no change in the testis. The rates of sterol synthesis were markedly increased in adrenal gland (42-fold) and ovary (2.7-fold) but not in testis. In the presence of normal levels of endogenous plasma lipoproteins, administration of adrenocorticotropin (ACTH) gel did not alter the rates of sterol synthesis in the adrenal gland but did essentially double the tissue content of cholesterol esters. In contrast, the administration of chorionic gonadotropin increased the rate of sterol synthesis in the ovary 4.1-fold while decreasing the cholesterol ester level from 7.32 to 1.15 mg/g and increased the rate of synthesis in the testis 10.4-fold under circumstances where there was no decrease in ester content. In the ovary, the effect of the simultaneous administration of 4-aminopyrazolo[3,4-d]pyrimidine and chorionic gonadotropin was additive, resulting in a 6- to 7-fold increase in the rate of sterol synthesis and a still further decrease in tissue cholesterol ester content. In 4-aminopyrazolo[3,4-d]pyrimidine-treated animals, the continuous infusion of 20 mg of cholesterol carried in high density lipoprotein over 42 h increased the cholesterol ester content of the adrenal gland from 1.4 to 18.8 mg/g and suppressed sterol synthesis from 381 to 29 nmol/g/h. In contrast, the infusion of an identical amount of cholesterol carried in low density lipoprotein caused only a slight increase in the cholesterol ester content and a modest suppression of synthetic activity. Similar results were found in ovary and testis. Finally, in these same groups of rats the increase in the plasma corticosterone, progesterone and testosterone levels was 6- to 12-fold higher with infusion of high density lipoprotein than with low density lipoprotein. It is concluded that lipoprotein cholesterol, rather than cholesterol newly synthesized in the glands, is the major substrate for the production of sterol hormones in the adrenal gland, ovary, and testis of the rat. Furthermore, these three endocrine glands preferentially take up and utilize high density lipoprotein cholesterol rather than low density lipoprotein cholesterol.

Recent studies have demonstrated that when circulating levels of cholesterol carried in various lipoprotein fractions are markedly reduced there are significant increases in the rates of de novo sterol synthesis in many tissues. For example, in the rat when chylomicrons are diverted from the circulation by cannulation of the lymph duct draining the gastrointestinal tract the rate of cholesterol synthesis in the liver increases approximately 3-fold (1). Furthermore, the rates of [1-14C]-acetate or [3H]water incorporation into digitonin-precipitable sterols increases 2- to 35-fold in nonhepatic tissues, such as small intestine, colon, ovary, kidney, lung, skin, spleen, adrenal gland, and adipose tissue, under circumstances where the plasma total cholesterol level is reduced to approximately 5 mg/dl by the administration of 4-aminopyrazolo[3,4-d]pyrimidine (APP) over a 3-day interval (2, 3). Similar striking increases in the levels of 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate-limiting enzyme in the cholesterol biosynthetic pathway, have been shown in at least the kidney, lung, and adrenal gland of animals treated in a similar fashion (4, 5). Restoration of circulating levels of the various lipoproteins results in a return of synthetic rates in these same tissues to the lower levels characteristic of the untreated rat. Thus, the intravenous administration of chylomicrons of rat origin suppresses the rate of hepatic cholesterol synthesis (1, 6, 7) while administration of a mixture of high and low density lipoproteins obtained from rat plasma inhibits sterol synthesis in the nonhepatic tissues of the APP'-treated animal (3). Such findings are consistent with the view that the rate of sterol synthesis in the liver and in a number of other tissues is regulated by the concentration of various lipoproteins circulating within the plasma.

The infusion of specific lipoprotein fractions of either rat or human origin into such animal preparations has led to the identification of important differences in the response of the sterol synthetic pathway in various tissues to different lipoproteins. Thus, the infusion of human LDL into APP-treated rats suppresses the rate of sterol synthesis in tissues such as the intestine, kidney, lung, and skin (3), suggesting that these tissues possess the LDL transport system described in detail in the fibroblast (8, 9). The infusion of chylomicrons, in contrast, into animals with normal circulating levels of HDL and LDL suppresses cholesterol synthesis only in the liver (10), indicating that only the hepatocyte possesses the high velocity transport system responsible for cellular uptake of chylomicron remnants (11-13). Regulation of synthesis in the adrenal gland and ovary of the rat differs significantly from regulation of either the liver or these nonendocrine, nonhepatic tissues in that the rates of sterol synthesis are most...
sensitive to the circulating levels of plasma HDL (3, 5, 14), suggesting that these two tissues possess yet a third type of lipoprotein transport mechanism. This possibility also is supported by the recent observation that uptake of radiolabeled cholesterol into slices of rat adrenal gland is faster from HDL than from LDL and, further, that uptake of HDL cholesterol is stimulated by the administration of ACTH (15).

Because of the marked quantitative and qualitative differences that have been observed in the regulation of cholesterol synthesis in these endocrine glands the three groups of studies reported here were undertaken. The first set of investigations deals with the relative importance of serum cholesterol in the maintenance of tissue cholesterol levels and in the regulation of rates of DPS synthesis in the adrenal gland, ovary, and testis. The second group of experiments compares the effect of the administration of the appropriate trophic hormones on rates of cholesterol synthesis and tissue cholesterol content in these same three endocrine glands under circumstances where the level of available plasma cholesterol was kept constant. In the final group of studies the relative importance of cholesterol carried either in HDL or LDL as effectors of the rates of DPS synthesis in the adrenal gland, ovary, and testis was assessed.

### Experimental Procedures

**Chemicals and Drugs**—4-Aminopyrazol[3,4-d]pyrimidine, cycloheximide, and octanoic acid were obtained from Sigma. Adrenocorticotropic (ACTH) gel (40 units/ml) came from Armour and human chorionic gonadotropin (500 units/ml) was from Ayerst. Aminoglutethimide was a gift from CIBA-GEIGY. [1-14C]Octanoate, [4-14C]cholesteryl olate, and [5,6-3H]cholesterol were from New England Nuclear and [3H]cholesterol was from Amersham.

**Lipoprotein Preparations**—Pooled plasma samples were obtained from normal human subjects fasted overnight or from rats weighing 250 to 450 g and maintained on a low cholesterol, low fat diet of rat chow (Ralston Purina). In order to minimize contamination of the specific label by lipoprotein fractions, the LDL fractions utilized in these studies were harvested in the density range of 1.020 to 1.050 g/ml while the HDL fractions were obtained in the density range of 1.060 to 1.230 g/ml. As previously described, these fractions were dialyzed against several changes of isotonic sodium chloride solution prior to administration to the animals (3).

**Animal Preparations**—The male and female Sprague-Dawley derived rat strains were studied in Charles River. When received in the laboratory, the animals weighed 125 to 150 g and were housed in colony cages in an isolated room with alternating periods of light (1500 to 0300 h) and dark (0300 to 1500 h) and allowed free access to Formula chow diet from Ralston Purina and water for 2 weeks prior to utilization in specific experiments.

Since the purpose of these studies was to evaluate the effect of either the lowering of serum cholesterol levels by means of APP administration or driving hormone production by the administration of ACTH or hCG on sterol synthesis in three endocrine glands, preliminary studies were necessary in order to establish the time course for drug administration that would give optimal effects. Administration of ACTH gel over 4 days resulted in a near doubling of the sterol content of the testis (15) and was used for the studies shown diagrammatically in Fig. 1. As previously described, the rates of incorporation of various 14C-labeled substrates, e.g., glucose, acetate, octanoate, pyruvate, etc., into DPS in these studies averaged approximately 120 oscillations/min and other nonhepatic tissues, it was used in the current studies (16, 17). Third, since rates of sterol synthesis were assayed in these studies by measuring the rate of incorporation of [1-14C]octanoate into DPS, it was conceivable that the rates would be underestimated to the extent that some of the newly synthesized cholesterol was further metabolized to steroids that were not digitonin-precipitable. To evaluate this possibility, experiments were performed in which testes, ovary, and adrenal gland from control animals and from animals treated with ACTH, hCG, and APP were incubated without inhibitors and in the presence of either aminoglutethimide (0.625 mg/ml) or cycloheximide (0.20 mg/ml) to block the further metabolism of cholesterol. In the presence of these inhibitors, absolute incorporation rates were somewhat higher in ovary but were essentially the same in adrenal gland and testis. Furthermore, the relative effects of APP or hCG administration were the same: thus, APP administration en...
hanced DPS synthesis in the adrenal gland 103- and 90-fold and in the ovary 7.0- and 8.0-fold, while hCG administration enhanced sterol synthesis in the ovary 4.0- and 5.1-fold in the absence and in the presence of the inhibitors, respectively. Since inclusion of the inhibitors did not significantly alter the experimental findings, they were not routinely included in the incubation media in subsequent experiments. Thus, on the basis of these preliminary experiments, the following protocol was adopted. All animals were killed by decapitation at 0900 h at the completion of the 4-day experimental period (Fig. 1) and the various tissues were immediately removed and placed in cold 150 mM NaCl solution. Slices of most tissues 0.8 mm thick were prepared using a mechanical tissue slicer while slices of similar thickness were prepared by hand from the ovary and adrenal gland. The testis was decapsulated and minced using the mechanical tissue slicer. Three hundred milligrams of all tissues except the ovary and adrenal gland were placed in 25-ml center-well incubation flasks; the two ovaries or adrenal glands obtained from individual animals were sliced, weighed, and placed in a single flask. In all cases, the incubation flask contained 5.0 ml of Krebsbicarbonate buffer equilibrated at 0°C with 95% O_2 and 5% CO_2, 5 μmol of sodium octanoate labeled with 5 μCi of [1-14C]octanoate, and 20 μmol of glucose. After flushing with 95% O_2, 5% CO_2, the flasks were sealed with rubber stoppers and incubated for 90 min in a metabolic incubator at 37°C.

**Chemical Methods**—At the completion of the incubations, radio-labeled CO_2 was trapped in hyamine hydroxide placed in the center well of each flask. Fifteen milliliters of alcoholic KOH solution (6 ml of 50% KOH added to 94 ml of absolute ethanol) and 0.5 mg of carrier cholesterol were added to each flask and the contents were saponified on a steam bath. The sterols were then extracted, precipitated as the digitonide, and assayed for 14C content (1, 16). The radioactivity in the DPS was then divided by the specific activity of the [1-14C]-octanoate, the time of incubation (1/2 h), and the weight of tissue in the flask and multiplied by factors of 4 (to convert nanomoles of sterols into sterol units) and 1.5 (to correct for loss of 33% of the 14C as [1-14C]CO_2 during the synthesis of DPS from [1-14C]acetyl CoA). This gives the rates of incorporation of acetyl-CoA units, i.e. C_2 units, into DPS/g of tissue/h (nanomoles/g/h) (16). A similar calculation was utilized for determining rates of oxidation of C_2 units into CO_2 except that the factor of 1.5 was omitted; these rates are given as the micromoles of acetyl-CoA oxidized/g of tissue/h (micromoles/g/h) (16). Total cholesterol concentration in plasma was measured using a modification of the Liebermann-Burchard method (19). The tissue content of unesterified and esterified cholesterol was measured using a modification of a previously described method (19). Tissue was extracted with chloroform:methanol (2:1, v/v) and the free and esterified sterols were separated on columns (30 cm). Plasma corticosterone and progesterone levels of 52 ± 18 ng/dl and 23.3 ± 8.2 ng/ml, respectively, while the testosterone level in the male control rats equaled 3.2 ± 1.4 ng/ml. In the animals treated with APP the three hormone levels decreased significantly and equaled 24 ± 3 μg/dl, 7.6 ± 3.2 ng/ml, and 0.2 ± 0.0 ng/ml, respectively.

The second group of experiments was designed to examine the effects of administration of the appropriate trophic hormones on cholesterol metabolism in the adrenal gland, ovary, and testis under circumstances where normal circulating levels of plasma lipoprotein cholesterol were present. The effect of ACTH treatment is summarized in the first three columns of Table II. The administration of this hormone clearly had no effect on the rate of DPS synthesis in any of these three endocrine tissues including the adrenal gland. In addition, there was no significant alteration in the concentration of unesterified or esterified cholesterol in the ovary or testis but the cholesterol ester content of the adrenal gland nearly doubled. To be certain that these findings in the adrenal gland were not the result of the administration of ACTH (10 units) every 24 h, another experiment was undertaken where 5 units of ACTH gel was administered subcutaneously every 12 h for 4 days. The results of this experiment were qualitatively identical to those summarized in Table II, including the rise in cholesterol ester content observed in the adrenal gland. The data shown in the three columns on the right side of Table II summarize the results obtained in animals receiving hCG treatment. Cholesterol metabolism in the adrenal gland was unaffected by administration of this trophic hormone. However, DPS synthesis increased 4.1- and 10.4-fold, respectively, in the ovary and testis. In the ovary, this increased rate of synthesis was associated with a marked decrease in the
Effect of APP administration on rates of sterol synthesis and levels of unesterified and esterified cholesterol in seven different tissues of the rat

Both male and female rats were treated as described in the protocol shown in Fig. 1; all animals were fasted during the 4-day experimental period. Control (CONT) animals were injected with only buffer solution while the experimental groups received daily injections of APP (20 mg/kg of body weight). None of these animals received the intravenous infusion shown in Fig. 1. After 4 days, the animals were killed at the mid-dark point of the light cycle and the intestine, lung, kidney, skin, adrenal gland, and ovary were taken from the female rats while only the testis was used from the male animals. Tissue slices were prepared and rates of C flux into DPS and into CO were assayed. In addition, the levels of unesterified and esterified cholesterol were measured in each organ. The values shown in this table represent the means ± 1 S.E. for five animals in each group. The asterisks indicate values in the APP-treated rats that differed significantly at the p < 0.05 level from the appropriate control (CONT) values.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>APP</th>
<th>CONT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>44.6 ± 4.9</td>
<td>43.5 ± 5.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.4 ± 0.4</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>Skin</td>
<td>34.5 ± 4.3</td>
<td>34.0 ± 3.7</td>
</tr>
<tr>
<td>Adrenal Gland</td>
<td>2.1 ± 0.2</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>Ovary</td>
<td>1.9 ± 0.1</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Testis</td>
<td>1.3 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
</tbody>
</table>

unesterified and esterified cholesterol content of the gland to 62 and 16% of control values, respectively. In contrast, unesterified and esterified cholesterol in the testis increased by a small absolute amount. In these experiments, the animals administered ACTH had no significant increase in circulating corticosterone levels, whereas the female rats that received hCG elevated their plasma progesterone levels from 5.7 ± 2.0 to 18.1 ± 4.1 μg/ml. It should also be emphasized that the total plasma cholesterol levels found in these various experimental groups of animals were all within the normal range of 52 to 60 mg/dl at the termination of the experiment. Significantly increased organ weights were observed in the adrenal glands following APP or ACTH treatment and in the ovaries following hCG treatment. Under such conditions, normalizing the data to a unit weight, as in Tables I and II, may produce artifactual differences between experimental and control animals. Therefore, these data were recalculated and, in the rates of DPS synthesis and the cholesterol content of the whole glands are shown. Clearly, after comparing the data in Tables I and II with those in Table III, it is apparent that the observed qualitative differences seen between experimental and control groups were maintained with one exception: the unesterified cholesterol content in the ovary from hCG-treated animals was slightly increased when expressed per organ but was slightly decreased when expressed per unit weight. Notably, depression of plasma cholesterol levels with APP enhanced DPS synthesis 84-fold in the pair of adrenal glands and 2.2-fold in the ovaries while hCG administration increased the rate of synthesis 9.4-fold in the ovary but ACTH was without effect in the adrenal gland. In a third set of experiments, both APP and hCG were administered simultaneously to male and female rats so as to deprive the ovary and the testis in these animals of circulating lipoprotein cholesterol while at the same time driving hormone output from these tissues. The results obtained in the testis were similar to those obtained with hCG treatment alone. The rates of sterol synthesis increased from 4.8 nmol/g/h in the control animals to 25.4 nmol/g/h in the APP/hCG-treated group and the mean tissue content of unesterified and esterified cholesterol increased from 2.41 to 10.2 mg/g and from 0.09 to 0.51 mg/g, respectively. In the ovary, the effect of administering hCG and APP simultaneously was approximately equal to the sum of the effects seen when each of these drugs was administered individually. Thus, the rates of sterol synthesis in animals treated either with APP or hCG increased by 408 and 290 nmol/g/h, respectively, while the rate of sterol synthesis increased by 793 nmol/g/h in the APP/hCG-treated animals. In addition, the tissue content of cholesterol esters in the ovary averaged 4.86 mg/g in the untreated control group, 2.41 mg/g in the APP'-treated group, and 0.96 mg/g in the hCG-treated group but was only 0.32
mg/g in the APP/hCG-treated animals.

The enhanced rates of sterol synthesis seen in the endocrine glands after reduction of plasma cholesterol levels with APP treatment suggested that one or more of the circulating lipoproteins normally suppressed sterol synthesis in the tissues in the intact animal. To examine this possibility more closely, various rat and human lipoprotein fractions were continuously infused intravenously over a 42-h period into rats that had been treated with APP to lower their levels of endogenous lipoprotein cholesterol to ~5 mg/dl. At the end of the infusion period, plasma cholesterol levels, rates of DPH synthesis, and tissue levels of unesterified and esterified cholesterol were assayed. Table IV summarizes the effect of infusing 20 mg of HDL cholesterol or LDL cholesterol of human origin on rates of sterol synthesis and tissue cholesterol content in the adrenal gland of female rats. The APP-treated rats receiving no lipoprotein (Column 2) demonstrated the expected decrease in plasma cholesterol concentration and tissue cholesterol content and the expected increase in the rate of sterol synthesis when compared to the untreated control group (Column 1). The continuous infusion of human HDL caused an increase in the steady state plasma cholesterol concentration from 3.6 to 50 mg/dl and suppressed the rate of sterol synthesis in the adrenal gland from 381 to 28.9 nmol/g/h. These changes were associated with a rise in the unesterified cholesterol content of the tissue from 2.38 to 4.11 mg/g and a marked increase in the cholesterol ester content from 1.41 to 18.82 mg/g. In contrast to these findings, even though the continuous infusion of an equal amount of cholesterol carried in LDL resulted in a slightly higher average plasma cholesterol concentration (68 mg/dl), there was far less accumulation of unesterified and esterified cholesterol in the adrenal tissue and significantly less suppression of the rate of sterol biosynthesis (Column 4).

Utilizing the same experimental protocol, these apparent differences in the effects of HDL and LDL were further explored by the continuous infusion of varying amounts of human or rat HDL and human LDL into a series of rats.

**TABLE IV**
The effect of infusing equal amounts of HDL cholesterol and LDL cholesterol on rates of sterol synthesis and tissue cholesterol content in the adrenal glands of APP-treated female rats

As shown diagrammatically in Fig. 1, all animals in this study were fasted during the 4-day experimental period and each was fitted with an indwelling tail vein catheter. One group of rats was injected intraperitoneally with buffer solution alone (Column 1) while three other groups were administered APP (Columns 2, 3, and 4) daily. Continuous intravenous infusions (1.5 ml/h) were begun in all groups 42 h prior to the termination of the study. Two groups of animals received only the glucose/electrolyte solution (Columns 1 and 2) while the other two groups received this solution containing either HDL (Column 3) or LDL (Column 4) isolated from human plasma. In both of these latter groups a total of 20 mg of lipoprotein cholesterol was infused over the 42-h period. This table shows the plasma cholesterol levels and the rates of sterol synthesis and tissue cholesterol content in the adrenal gland at the time the various groups of animals were killed. The values represent the means ± 1 S.E. for 7 to 10 animals in each group. The asterisks indicate those values that differed significantly at the p < 0.05 level when compared to the APP-treated control group shown in Column 2.

![Fig. 2. The effect of infusing varying amounts of HDL and LDL on rates of sterol synthesis and tissue cholesterol levels in the adrenal glands of female rats.](http://www.jbc.org/) Utilizing the experimental protocol shown in Fig. 1, animals treated with APP were infused with varying amounts of cholesterol carried in either HDL or LDL over a 42-h period. In this figure the rate of C~4~ flux into DPH (left panel) and the tissue cholesterol levels (right panel) found in the adrenal gland when the animals were killed are plotted as a function of the final plasma total cholesterol concentration. HDL and LDL obtained from human plasma and HDL from rat plasma were utilized in these studies. Each point represents the results obtained in an individual animal. Since the results obtained with the rat and human HDL were undistinguishable they are shown as a single curve.
The studies shown in this table were undertaken as described in detail in the legend to Table IV except that male rats were injected with both APP and hCG daily during the 4-day experimental protocol.

**Table V**
The effect of infusing equal amounts of HDL cholesterol and LDL cholesterol on rates of sterol synthesis and tissue cholesterol content in the ovaries of APP-treated rats

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>CONTROL</th>
<th>APP</th>
<th>hCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Cholesterol (mg/dl)</td>
<td>66.7</td>
<td>66.4</td>
<td>65.9</td>
</tr>
<tr>
<td>C. Flux into DPS (nmol/g/h)</td>
<td>2.43</td>
<td>2.54</td>
<td>2.63</td>
</tr>
<tr>
<td>C. Flux into CO (nmol/g/h)</td>
<td>0.61</td>
<td>0.62</td>
<td>0.63</td>
</tr>
<tr>
<td>Cholesterol Ester (mg/dl)</td>
<td>2.05</td>
<td>2.06</td>
<td>2.07</td>
</tr>
</tbody>
</table>

**Table VI**
The effect of infusing equal amounts of HDL cholesterol and LDL cholesterol on rates of sterol synthesis and tissue cholesterol content in the testes of rats treated with both APP and hCG

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>CONTROL</th>
<th>APP</th>
<th>hCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Cholesterol (mg/dl)</td>
<td>51.9</td>
<td>51.5</td>
<td>51.1</td>
</tr>
<tr>
<td>C. Flux into DPS (nmol/g/h)</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>C. Flux into CO (nmol/g/h)</td>
<td>0.61</td>
<td>0.62</td>
<td>0.63</td>
</tr>
<tr>
<td>Cholesterol Ester (mg/dl)</td>
<td>2.05</td>
<td>2.06</td>
<td>2.07</td>
</tr>
</tbody>
</table>

**DISCUSSION**

These studies provide additional support for our previous contention that DPS synthesis in the endocrine glands of the rat is controlled by regulatory mechanisms that are uniquely different from those that operate in the regulation of synthesis in the liver and in nonhepatic, nonendocrine tissues and suggest that the membranes of these endocrine cells possess a transport mechanism that operates to translocate HDL into...
the cell. Several such lipoprotein transport systems have now been described and account for the particular characteristics of regulation of sterol synthesis seen in specific tissues. In general, the rate of cholesterol synthesis seen in a particular cell type is determined by the balance of the rate of input of cholesterol into the cell from the outside relative to the needs of the cell for sterol for the synthesis of specialized products or for structural or secretory purposes. As shown diagrammatically in Fig. 4, cholesterol entry into cells occurs principally by two mechanisms, the passive diffusion of monomolecular cholesterol through the cell membrane (Pathway 1) or the uptake of lipoproteins by endocytic processes (Pathway 2). The unidirectional rate of cholesterol diffusion into or out of the cell is determined by the product of the passive permeability coefficient for the sterol molecule and the chemical activity coefficient for the sterol molecule and the chemical activity coefficient for cholesterol across the cell membrane (25). Thus, experimental conditions can be established where net molecular cholesterol movement is out of the cell, e.g. when isolated cells are incubated with substances that bind or take up cholesterol, or into the cell, e.g. when cells are incubated with more polar derivatives of cholesterol. Uptake of lipoproteins, however, appears to be quantitatively far more important in the net delivery of cholesterol into the cell. In the instances where the kinetics of this process are known, the rate of uptake is a saturable process involving a finite number of sites on the cell membrane (8, 9, 13). After entry into the cell through endocytosis, the sterol esters carried in the lipoprotein are hydrolyzed to free cholesterol and a portion of this may then be re-esterified and deposited in an intracellular pool of cholesterol esters (9). Thus, as is apparent in Fig. 4, there are potentially three major sources of cholesterol available to the cell to meet its metabolic needs; these include the hydrolysis of stored cholesterol esters (Pathway 4), cholesterol delivered directly from the uptake of lipoproteins into the cell (Pathway 2), and cholesterol synthesized de novo from acetyl-CoA (Pathway 3). Similarly, there are potentially three points of regulation of this system including alterations of the rate of ester hydrolysis (z), the rate of lipoprotein transport into the cell (y), and the rate of cholesterol synthesis (x). While such a model presumably can be applied to the regulation of cholesterol balance in almost any cell in the body, there is currently sufficient information to suggest that there are unique differences in the characteristics of each of these processes in different specialized tissues.

One group of tissues appears to reflect the situation worked out in detail in the fibroblast (8, 9, 26). As described in detail by Brown and Goldstein (27), these cells transport LDL by a relatively low velocity, saturable transport system; coincident with the entry of LDL cholesterol into the cells there is suppression of intracellular cholesterol synthesis (x) and, equally important, suppression of further transport of LDL into the cell (y). As a consequence, there are marked reciprocal changes between the rates of sterol synthesis in the cell and the availability of LDL cholesterol under circumstances where the level of cholesterol ester remains relatively unchanged. In the present studies, when the circulating levels of all lipoproteins in the plasma were markedly reduced by APP administration, sterol synthesis significantly increased in mid-gut, lung, kidney, and skin yet there was essentially no change in the levels of either unesterified or esterified cholesterol in these tissues (Table I). This finding, coupled with the observation that synthesis in these same tissues is suppressed by infusion of LDL into similarly treated animals (3) strongly suggests that the fibroblast model applies to at least this group of nonhepatic, nonendocrine tissues.

Regulation of sterol synthesis in the liver differs in several significant ways from regulation in these particular tissues. Partially metabolized chylomicrons or "remnants" are taken up into the liver cell by a high velocity, saturable transport system (11-13). In contrast to LDL transport in the fibroblast, the rate of hepatic uptake of remnants is not affected by the entry of lipoprotein cholesterol into the cell, i.e. there is no regulation of the transport step at y in Fig 4 (17). Thus, when chylomicrons are present in the circulation there is rapid clearance of the remnants into the liver with accumulation of the cholesterol esters and reciprocal suppression of cholesterol synthesis (6). Currently, there is no experimental evidence indicating that the hepatocyte membrane possesses specific transport mechanisms for the uptake of either HDL or LDL.

The rate of hepatic cholesterol synthesis also is directly related to the rate of sterol loss from the hepatocyte during lipoprotein synthesis (29), bile acid synthesis (30-32), or cholesterol secretion into bile. Thus, for example, when the entry of remnant cholesterol into the liver cell is kept constant, the rate of cholesterol synthesis varies directly with the rate of bile acid synthesis (33).

The current studies suggest that the adrenal gland, ovary, and testis of the rat behave very much like the liver with respect to regulation of cholesterol synthesis except that the cell membranes contain a transport system primarily directed at the translocation of HDL rather than chylomicron remnants. Either limiting the availability of lipoprotein cholesterol or enhancing the loss of cholesterol from the cells by appropriate trophic hormone administration leads to depletion of the pool of sterol esters and a compensatory increase in de novo cholesterol synthesis.

The interplay of these two processes in the intact animal, however, is complex. When availability of plasma lipoproteins is limited by treatment with APP, the synthesis of DPS increased by a factor of 42.4, 2.7, and 0.8 in the adrenal gland, ovary, and testis, respectively (Table I). In contrast, when plasma cholesterol levels were normal, but hormone production was driven with either ACTH or hCG, the synthesis of DPS increased by a factor of 0.6, 4.1, and 10.1 in the adrenal gland, ovary, and testis, respectively (Table II). This latter observation in the ovary is compatible with the findings of Kovanen et al. that ovarian HMG-CoA reductase activity increases markedly with pregnancy or luteinizing hormone administration in the rabbit (34). Initially, these data suggested that DPS synthesis in the adrenal gland was primarily regulated by the availability of lipoprotein cholesterol while synthesis in the testis and, to a lesser degree, in the ovary was primarily regulated by the rate of testosterone or progesterone.
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secretion. However, measurement of circulating hormone levels revealed that the concentrations of progesterone and testosterone were very low in the APP-treated animals, suggesting that there was little synthesis of these two hormones under this experimental circumstance. Presumably, therefore, there was little need to increase cholesterol synthesis in these glands to compensate for the diminished rate of uptake of plasma lipoprotein cholesterol. When hormone output was maintained by administration of exogenous ACTH and hCG, it became apparent that the synthesis of DPS in all three glands increased markedly when the lipoprotein source of cholesterol was removed. Thus, like the liver, the rate of DPS synthesis in these endocrine glands is dictated by the balance between the rate of lipoprotein uptake on the one hand and the rate of hormone synthesis on the other.

The storage pool of cholesterol esters (Fig. 4) varies in an inverse fashion with the rate of cholesterol synthesis as the uptake and output of sterols are systematically altered even though the absolute level of total cholesterol in these three endocrine tissues is very different. It should be emphasized that cells actively engaged in hormone production make up the majority of the cells in the adrenal gland, a lesser proportion of the cells in the ovary, and only a very small part of the cells in the testis. Since the total cholesterol content of most nonendocrine cells equals approximately 2 to 3 mg/g wet weight of tissue, it is not surprising that the cholesterol content of the adrenal gland (40 mg/g), ovary (11 mg/g), and testis (2.5 mg/g) decreases as the proportion of nonendocrine cells increases. Despite these marked differences, however, the pool of cholesterol esters generally decreased when uptake of lipoprotein cholesterol was reduced by APP treatment or when increased hormone synthesis was induced by hCG administration and, in the ovary, for example, these effects were additive. Cholesterol ester content in this gland decreased from the control level of 4.86 to 2.41 mg/g with APP treatment, to 0.96 mg/g with hCG administration, and to 0.32 mg/g when both drugs were given together. Thus, the cholesterol ester content of these glands, like the liver, is also dictated by the balance between the transport of lipoprotein cholesterol into the cell and the rate of loss of sterol from the cell.

In two previous studies, we have reported that HDL is a more potent regulator than LDL of the synthesis of DPS in the adrenal gland (3, 14) and in a similar study Balasubramaniam et al. (5) also found that much higher plasma concentrations of LDL than HDL were required to produce comparable suppression of adrenal HMG-CoA reductase activity. These findings are confirmed and extended in the present study (Pathway 2, Fig. 4) and that cholesterol derived from this lipoprotein suppresses intracellular cholesterol synthesis, is partially stored as cholesterol esters, and is utilized as substrate for hormone production.

Finally, five additional points can be made concerning the characteristics of this HDL cholesterol transport system. First, if HDL uptake occurs by mechanisms analogous to those described for LDL in the fibroblast and chylomycin remnants in the hepatocyte, then uptake presumably involves interaction between the lipoprotein and a specific number of receptor sites on the cell membrane. Saturation kinetics have been reported for uptake of radiolabeled cholesterol from HDL in rat adrenal gland in vitro (15) and we have shown similar saturation kinetics for the uptake of cholesterol mass in both the adrenal gland and ovary in vivo.3 Second, while this transport system clearly manifests the highest rates of uptake for HDL cholesterol, it is not specific for this lipoprotein fraction since LDL exerts similar metabolic effects, at least in the adrenal gland, when plasma concentrations are raised to sufficiently high levels (3, 5). Third, several lines of evidence suggest that in the adrenal gland ACTH may actually stimulate the transport of lipoprotein cholesterol into the cell as well as exert its well known effects within the cell on the conversion of cholesterol to adrenal steroids (35-37). In the present study, for example, ACTH administration doubled the content of cholesterol esters in the gland (Table I) under circumstances where the cholesterol ester content was already high and where synthesis of DPS was nearly totally suppressed. Gwynne et al. (15) also have reported that the uptake of radiolabeled cholesterol by adrenal slices from HDL, but not from LDL, is stimulated by ACTH administration to the donor animals. Whether the uptake rate of HDL cholesterol by the ovary and testis also is stimulated by hCG administration remains to be explored. Obviously, there would be a major physiological advantage to the animal if ACTH or hCG stimulated the uptake rate of lipoprotein cholesterol into the endocrine glands at the same time that these trophic hormones enhanced the rate of conversion of cholesterol to the appropriate product. Fourth, it is not known whether the HDL transport process in the adrenal gland, ovary, and testis is unique to the rat, which has predominantly HDL lipoproteins in its plasma, or is present in other species. Faust et al. (38) have reported the presence of specific LDL uptake in cultured Y-1 adrenal tumor cells obtained from the mouse and Chang and Ryan (39) have reported preliminary data on binding of HDL to porcine granulosa cells. Except for these studies, however, there is currently no information on the relative importance of these transport processes in other species. Fifth, the conclusion from these studies that lipoprotein cholesterol is the substrate for hormone synthesis in these three endocrine glands is consistent with previous reports utilizing the adrenal gland (40, 41) but is contrary to the usual interpretation of the data of Morris and Chaikoff (40) that most cholesterol used for androgen production in the testis is derived from intraglandular synthesis. However, this latter conclusion is probably not warranted since the specific activity values reported in this study primarily reflect the specific activity of the noninterstitial cells that make up the bulk of the tissue weight rather than the specific activity of the cholesterol pools present within the Leydig cells.

Acknowledgments—We would like to acknowledge the excellent technical assistance of Kathy Upham, Joan Thoson, Joyce Eckles, Visitacion Shine, and the skills of Nancy Mandell in the preparation of the manuscript.

3 Unpublished observations to be reported elsewhere.
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Relative importance of high and low density lipoproteins in the regulation of cholesterol synthesis in the adrenal gland, ovary, and testis of the rat.

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