Age-related decreases in tissue sterol acquisition are mediated by changes in cholesterol synthesis and not low density lipoprotein uptake in the rat

Eduard F. Stange and John M. Dietschy

Department of Internal Medicine, University of Texas Health Science Center at Dallas, Southwestern Medical School, Dallas, TX 75235

Abstract The present investigation compared plasma cholesterol levels and lipoprotein profiles, and absolute rates of sterol synthesis and low density lipoprotein (LDL) uptake in various organs of immature (4 weeks old) and mature (15 weeks) rats. The plasma cholesterol level and its distribution among the major lipoprotein density fractions were similar in both groups. Using [^{3}H]water as a substrate for measuring sterol synthesis in vivo, the content of newly synthesized cholesterol (^{3}H-labeled digitonin-precipitable sterols; [^{3}H]DPS) was several fold higher in all tissues of the young, compared to the old, rats when normalized per g of tissue. In contrast, whole-body [^{3}H]DPS content was identical at 29.5 and 29.3 μmol/hr in young and old rats, respectively, despite a 4.4-fold difference in body weight (102 vs. 453 g). The importance of different organs to total-body sterol synthesis remained similar with increasing age although the skin (11 vs. 24% of total) rather than the small bowel (15 vs. 8%) became the second most important organ after the liver (49 vs. 45%) in the older animals. When LDL uptake was determined in these same organs, using a constant infusion technique, the rates of clearance were higher only in the adrenal glands, adipose tissue, and skin of the young animals; whereas these rates were essentially the same in the liver and gastrointestinal tract, the two organs that are quantitatively most important for LDL catabolism. Even when these clearance rates were normalized to the whole organ or to 100 g of body weight, the differences in LDL uptake in the two age groups were minor compared to the major decrease in rates of cholesterol synthesis that were observed with aging. Finally, calculation of absolute rates of tissue cholesterol acquisition from both sources indicated that, in most organs, the majority of tissue cholesterol was derived from local synthesis rather than from LDL uptake in both age groups and that, with increasing age, total cholesterol acquisition decreased several-fold primarily as a consequence of the diminished rate of sterol synthesis. These studies demonstrate that with growth and aging in the rat there is a dramatic decrease in the rate of tissue cholesterol synthesis while the uptake of LDL-cholesterol remains essentially unchanged.—**Stange, E. F., and J. M. Dietschy.** Age-related decreases in tissue sterol acquisition are mediated by changes in cholesterol synthesis and not low density lipoprotein uptake in the rat. *J. Lipid Res.* 1984. **25:** 703–713.

Supplementary key words [^{3}H]water • cholesterol balance • growth

During the growth of an animal there is continuous need for cholesterol for the synthesis of new membranous structures so that, on average, about 1.5 g of sterol is accumulated in the body for every 1000 g of new tissue that is laid down (1). Even when the mature animal is no longer in a rapid growth phase, most cells continue to require a supply of cholesterol to meet continued needs for membrane synthesis (as in tissues like the intestine and bone marrow), membrane lipid turnover, and the synthesis of sterol products (as in the liver and endocrine glands). Such needs generally are met either by the synthesis of cholesterol within a given tissue or by the uptake of cholesterol from the extracellular environment through either molecular diffusion (2) or the receptor-dependent or -independent uptake of cholesterol carried in different classes of lipoproteins (1). Recently it has become possible to quantitate both of these processes in the various organs of intact animals.

All tissues manifest at least some synthetic ativity, although these rates may vary as much as 200-fold among the different organs (3). In different species, including man to the extent data are available, the highest rates of cholesterol synthesis (expressed per g wet weight) are usually found in organs such as liver, intestine, and the endocrine glands, while the lowest rates are found in tissues such as brain, striated muscle, and adipose tissue (1, 3, 4). Such rates are regulable in nearly all of the organs under circumstances where sterol flux across the cells of a particular tissue is changed. For example, increasing or suppressing the diffusion of molecular cholesterol into the intestinal epithelial cells decreases or enhances, respectively, the rates of cholesterol synthesis in these cells (5–7). Similarly, either increasing or decreasing the amount of cholesterol delivered to the hepatocyte in the chylomicron remnant also is associated with an appropriate reciprocal change in the rate of sterol synthesis by the liver (8, 9). Finally, limiting the availability

Abbreviations: LDL, low density lipoproteins; DPS, digitonin-precipitable sterols; rLDL, LDL from rat plasma; HMG. 3-hydroxy-3-methylglutaryl.

of low density lipoprotein (LDL)-cholesterol for transport into certain peripheral organs is, again, associated with an appropriate increase in the rate at which these various organs synthesize sterol (10–12).

Just as nearly every organ can synthesize cholesterol, these same tissues can also take up cholesterol carried in LDL by either receptor-dependent or -independent mechanisms. In animals such as the rat, rabbit, hamster, and man, receptor-dependent uptake accounts for from two-thirds to three-fourths of total LDL degradation from the plasma space (13). As in the case of cholesterol synthesis, the highest rates of LDL uptake (expressed per g wet weight) are found in organs like liver, intestine, and the endocrine glands, and the majority of this uptake is receptor-dependent (14, 15). Similarly, the lowest rates are seen in brain, muscle, and adipose tissue, and this minimal uptake appears to be mediated by a receptor-independent mechanism.

Thus, the generalization can now be made that cholesterol synthesis and the uptake of sterol carried in lipoproteins act in concert to meet the needs of a particular organ. Both processes occur at high rates in tissues requiring a great deal of cholesterol such as the cells of the intestinal crypts, liver, and endocrine glands while correspondingly lower rates for both processes are found in organs such as skin, muscle, adipocytes, and brain that require little cholesterol (12–15). Nevertheless, of these two processes, cholesterol synthesis is by far the most important. In so far as quantitative data are now available, for example, only about 2–30% of the cholesterol acquired by most organs comes from the uptake of LDL (13–16); the great majority comes from the de novo synthesis.

If cholesterol homeostasis in cells is to be maintained, then these processes of cholesterol acquisition must be regulable. Data obtained in a variety of isolated cells studied in vitro suggest that there is coordinated regulation of these various processes (17–19). Enhanced entry of cholesterol into the cells, for example, is associated with suppression of both cholesterol synthesis and LDL receptor activity, as well as with an increase in the rate of cholesterol esterification. Now, however, there is a variety of observations suggesting that such coordinated regulation does not take place in the differentiated tissues of live animals but, rather, that these processes are under independent regulation. For example, rates of hepatic cholesterol synthesis can be varied as much as 200-fold in some species without altering the rate of hepatic LDL uptake, the circulating levels of LDL-cholesterol, or whole-animal turnover of LDL-protein (13, 20). Similarly, altering the flux of molecular cholesterol across the absorptive cells of the intestine changes the rate of cholesterol synthesis in these cells as much as 10-fold, yet LDL uptake by these same cells remains essentially constant (7, 16). Thus, under most physiological circumstances that have been studied in vivo, a change in the flux of cholesterol into or out of a particular cell is met by a change in the rate of cholesterol synthesis in that cell rather than by a change in the rate of uptake of LDL. Only in extreme circumstances where changes in the rate of cholesterol synthesis cannot compensate (or are inhibited) for changes in cholesterol flux does the cell alter its rate of LDL transport (21, 22).

Another important model for studying these regulatory events is the growing animal. During the transition from rapid growth in the young animal to more stable weights in the adult, there must be marked changes in the need for cholesterol by every organ in the body. Since in most species there is essentially no accumulation of cholesterol in the various tissues nor changes in circulating levels of LDL during the transition from the rapid growth phase to maturity, it is apparent that there must be marked changes in the rates of cholesterol acquisition by the various organs during this transition.

The present studies were designed to examine these regulatory events. Using newer techniques, rates of cholesterol synthesis and LDL uptake were measured in vivo in the major tissues of young, rapidly growing rats and in much larger, more mature animals. From these measurements, it was determined that during the transition from rapid growth to maturity, the decreased needs for cholesterol in nearly every organ were met by a marked decrease in the rate of cholesterol synthesis in that organ while the rates of LDL uptake remained essentially constant.

MATERIALS AND METHODS

Animals

The male Sprague-Dawley-derived rats (CD(R) (SD)BR, Charles River Breeding Laboratories, Wilmington, MA) were kept in a room with alternating periods of light (1500–0300 hr) and darkness (0300–1500 hr) and were fed plain rat chow (Wayne Laboratory Animal Diets, Allied Mills, Inc., Chicago, IL) ad libitum for 10 days before being used in the experiments. At the time of the experiments the young (growing) and old (mature) animals were 30 and 89–103 days old and weighed approximately 100 and 450 g, respectively (**Table 1**).

Determination of sterol synthesis in vivo

As described previously in detail (3, 12), the rats were administered intravenously 50 mCi of [^{3}H]water between 0900 and 1100 hr (mid-dark period) and were killed exactly 1 hr later. The abdomen was opened and blood was drawn for the determination of plasma water specific

TABLE 1. Mean organ weights in the young and old rats

Tissue	Young Rats	Old Rats
	g wet weight	
A. Whole animal	101.6 ± 2.7	453.0 ± 6.7
B. Liver	4.7 ± 0.1	13.1 ± 0.6
C. Adrenal gland	0.01 ± 0.001	0.02 ± 0.002
D. Small bowel	6.5 ± 0.3	13.1 ± 0.8
E. Stomach	0.9 ± 0.1	1.8 ± 0.1
F. Colon	2.1 ± 0.1	4.8 ± 0.7
G. Lung	0.8 ± 0.03	1.6 ± 0.07
H. Spleen	0.5 ± 0.02	0.7 ± 0.06
I. Skin	15.4 ± 0.4	51.6 ± 2.2
J. Kidney	0.5 ± 0.01	1.4 ± 0.04
K. Testis	0.4 ± 0.01	1.5 ± 0.03
L. Carcass	55.2 ± 2.0	265.3 ± 4.8

This table lists the mean animal and organ weights for the experimental animals used in the studies shown in Figs.2 and 3. The carcass contained all the tissues not otherwise listed in this table. The values represent the means ± 1 SEM for six rats.

activity (23). The following organs were sampled and weighed: liver, adrenal gland, small bowel with contents, stomach, colon, lung, spleen, skin, kidney, testis, and carcass, which contained all of the other tissues not listed. In addition, medial thigh muscle and retroperitoneal fat also were sampled. Gastric and colonic contents were discarded. The entire small intestine, carcass, skin, and adrenal glands, as well as aliquots of the other tissues, were saponified in alcoholic KOH. The digitonin-precipitable sterols (DPS) were isolated and assayed for radioactivity as described (23).

Determination of tissue LDL clearance (uptake) in vivo

Homologous rat LDL (rLDL) was isolated as detailed previously (20) in the density range of 1.030–1.055 g/ml from donors fed a low cholesterol diet. The rLDL preparation was then labeled with [^{14}C]sucrose (24), dialyzed overnight against 0.9% NaCl, and filtered through a 0.22-μm Millipore filter immediately before use. The rLDL tissue clearance was measured during a constant infusion of [^{14}C]sucrose-labeled rLDL using a procedure validated recently in both hamster (14, 25) and rat (13). Groups of rats were fitted with femoral vein catheters and kept in restraining cages during the infusion period. Young and old rats received a priming dose of 500,000 and 1,000,000 dpm, respectively, of the same rLDL preparation and were then administered a constant infusion that delivered 17% of the initial bolus each hr to maintain a constant specific activity in plasma. This infusion rate was based on the whole body turnover rate of rLDL (20) and resulted in a stable plasma rLDL specific activity between 2 hr (70 ± 4 and 50 ± 1 dpm/μl in young and old animals, respectively) and 5 hr (73 ± 8 and 50 ± 2 dpm/μl, respectively) of the experimental period. Groups of animals were killed at these time points and the organs listed in Table 1 were removed, weighed, and sampled. Aliquots of these organs, along with the plasma samples, were saponified, neutralized, solubilized, and assayed for radioactivity (with quench correction) as described (14, 20). Although preliminary studies did not reveal any diurnal variation in LDL clearance, these measurements were also made at approximately the mid-dark phase of the light cycle.

Determination of the distribution of cholesterol in plasma lipoproteins

Pooled plasma from each group was anticoagulated with EDTA and equal volumes were adjusted to the densities of 1.006, 1.030, 1.055, 1.095, and 1.210 g/ml and centrifuged simultaneously at 165,000 *g* for 24 hr. The cholesterol concentration in the top 4 ml of each centrifuge tube, as well as in an aliquot of the plasma pool, was then measured colorimetrically (26).

Calculations

For the in vivo sterol synthesis experiments it was necessary to determine the mean specific activity of plasma water during the 1-hr experiment. This value was expressed as the dpm of ^{3}H per nmol of water and was calculated from the following equation (23):

$$\frac{(\text{dpm}^3\text{H/ml plasma})(1.09)}{(\text{nmol water/ml water})(0.92\text{ ml water/ml plasma})}.$$

The term 1.09 corrects the value found at the end of the 1-hr period to the mean plasma water specific activity during the experiment.

The rates of sterol synthesis (or newly synthesized sterol content) were calculated as the nmol of [^{3}H]water incorporated into DPS per hr per g of tissue (nmol/hr per g) using the following relationship (12):

$$\frac{(\text{dpm}[^3\text{H}]\text{DPS})}{(1\text{ hr})(\text{g tissue weight})(\text{sp act body water})}.$$

For the in vivo rLDL uptake experiments the tissue spaces at each time point during the constant infusion (2 and 5 hr) were determind by dividing the radioactivity in 1 g of each organ (dpm/g) by the steady state concentration of radioactivity in the plasma (dpm/μl); thus, the space of distribution had the units μl/g (27). Since the rLDL accumulates in each organ as a linear function of time (14, 15), the tissue clearance of rLDL was calculated from the slope of the best-fit linear regression curve of the relationship between the tissue space and time, and was expressed as μl/hr per g (14). Finally, when this clearance value was multiplied by the amount of rLDL-cholesterol in 1 μl of plasma, the absolute rates of rLDL-cholesterol uptake per hr per g of tissue were obtained.

The data represent means ± 1 SEM for the number of animals used in each study. The linear regression curves were fitted by the method of least squares. The statistical comparison was based on the Student's *t*-test.

RESULTS

In an initial set of experiments, plasma cholesterol levels and the lipoprotein-cholesterol concentration in various density fractions were measured in two groups of rats. One group was young and rapidly growing (30 days old, weighing 97 ± 4 g) while the second group contained more mature animals (89–103 days old, weighing 422 ± 6 g). As shown in **Fig. 1,** with increasing age there was no significant change in total plasma cholesterol levels between the young (66.3 mg/dl) and old (62.2 mg/dl) rats. In addition, the plasma cholesterol distribution among the different plasma lipoprotein density classes was similar in both groups of rats. Thus, these findings effectively eliminated age-related differences in lipoprotein profiles as a potential cause for changes in sterol synthesis or LDL uptake that might be found in these two groups of animals.

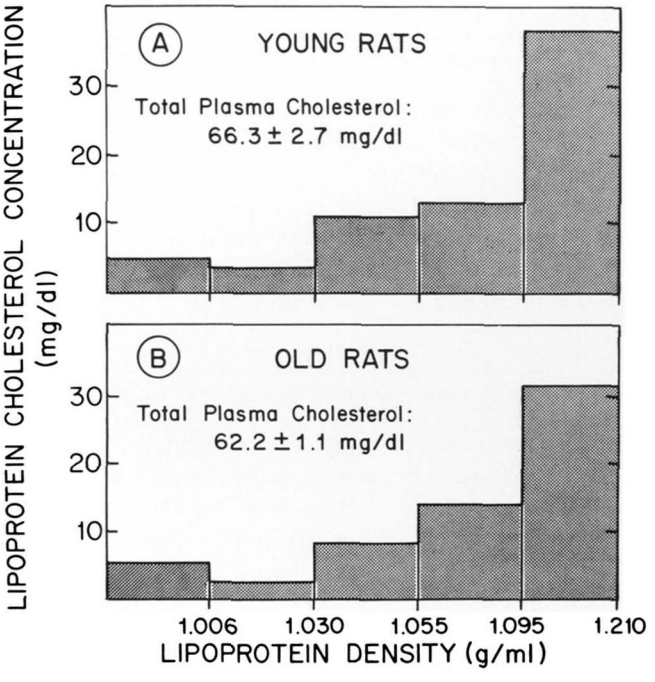

Fig. 1. Distribution of plasma cholesterol among five lipoprotein density fractions in young (panel A) and old (B) rats. Pooled plasma was adjusted to the densities of 1.006, 1.030, 1.055, 1.095, and 1.210 g/ml and centrifuged simultaneously at 165,000 *g* for 24 hr. The cholesterol concentration was then assayed in the top fractions and expressed as the plasma concentration (mg/dl) of the respective density class. The data represent the means of two separate pools of plasma from each age group. Total body weight was 97 ± 4 g and 422 ± 6 g for the young and old rats, respectively.

With this information available, the absolute rates of sterol synthesis were next determined in vivo in two similar groups of animals using [^{3}H]water. As is apparent in **Fig. 2,** the content of newly synthesized sterol found in 14 tissues of the two groups of animals gave a profile similar to that already reported in female rats weighing approximately 200 g (12). There were high levels of newly synthesized cholesterol in the liver, adrenal glands, and various portions of the gastrointestinal tract while much lower values were found in more peripheral tissues. However, although the relative distribution of synthetic activity was similar in the two groups of animals, the content of [^{3}H]DPS present in each g of tissue in the older animals (panel B) was signifiantly less than the values found in the actively growing, younger rats (panel A). Thus, the liver and small intestine, two of the most important organs for synthesis, manifested rates of synthesis in the older rats that were less than one-third of the rates found in the growing animals. There were even more marked differences observed in more peripheral tissues such as fat and muscle where the content of [^{3}H]DPS in the older rats was less than 15% of that seen in the young animals.

It should be noted that, in this type of study, there is transfer of some newly synthesized cholesterol from one organ to another, even within the 1-hr interval over which these rates were assayed (12). For practical purposes, all [^{3}H]DPS appearing in blood comes from the liver; hence, the content of [^{3}H]DPS in the blood (Fig. 2) in the young and old animals was proportional to the content of [^{3}H]DPS in the liver of these respective groups (12). Some of the [^{3}H]DPS in the blood is then taken up, by exchange or by net lipoprotein transport, and contributes to the content of [^{3}H]DPS in the different organs. Since the synthesis of cholesterol in the liver, and hence the content of [^{3}H]DPS in the blood, is so much lower in the older animals, a component of the lower content of [^{3}H]DPS in some of the peripheral tissues may be attributed to lower rates of uptake of [^{3}H]DPS from the blood rather than to lower rates of synthesis. However, these small sources of error in determining rates of cholesterol synthesis can be corrected for by utilizing previously published equations (12). When this is done, the absolute rates of synthesis occurring in vivo are still very much lower in the older animals, relative to the young rats, and amount to, for example, 158 versus 593 nmol/hr per g in the intestine, 3 versus 60 nmol/hr per g in the fat tissue, and 3 versus 21 nmol/hr per g in the skeletal muscle. Only in the adrenal gland are these corrections significant since <10% of the [^{3}H]DPS found in this organ in the rat is derived from synthesis; most is taken up from circulating high density lipoproteins (12, 28).

When each of these values was multiplied by the appropriate organ weight (Table 1), the [^{3}H]DPS content of each organ, as well as the rate of whole-animal cho-

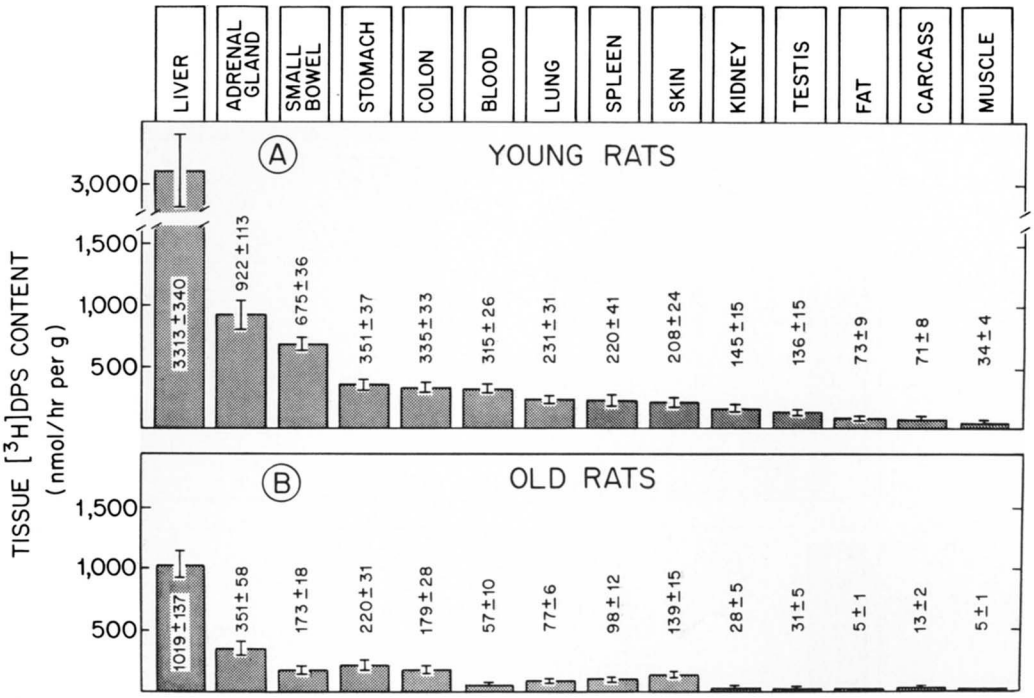

Fig. 2. Content of newly synthesized sterol in the various tissues of young (panel A) and old (B) rats. All animals were killed 1 hr after the intravenous administration of [^{3}H]water and the tissues were removed, weighed, and assayed for their content of [^{3}H]DPS. These contents were expressed as the nmol of [^{3}H]water incorporated into DPS per hr per g of tissue. The bulk of the adipose tissue (fat) and skeletal muscle remained with the residual carcass except for the small aliquots that were used to assay for [^{3}H]DPS content. The mean body weights of these groups are given in Table 1. The data represent the means ± 1 SEM for results obtained in six animals of each age group. The values are significantly different for every organ between the two groups at the $P < 0.05$ level.

lesterol synthesis, was obtained for the two groups of rats. As shown in **Fig. 3,** the absolute rate of [^{3}H]water incorporation into DPS equaled 29.5 and 29.3 μmol/hr in the young and old rats, respectively. These values compared to a previously reported value of 31.6 μmol/hr found in animals of intermediate size between the young and old rats used in the present study (3). Thus, when normalized to a constant body weight of 100 g, the rate of [^{3}H]water incorporation into sterols by the whole animal dropped dramatically from 29.1 to 15.8 to 6.5 μmol/hr per 100 g body weight as the animals aged and reached mean weights of 102 g, 200 g, and 453 g, respectively.

The relative contribution of each organ to cholesterol synthesis in the whole animal is similar in the young and old rats, as is also apparent in Fig. 3. For example, of the total [^{3}H]DPS formed, 49% and 45%, respectively, was found in the liver of the young and old rats. Similar contents of [^{3}H]DPS were found in many of the extrahepatic tissues. The two important exceptions were the intestine and skin. With aging, the amount of newly formed sterol found in the small bowel decreased from 15% of the total to only 8%, while that found in skin nearly doubled from the 11% seen in the young animals to the 24% found in the old rats.

With the rates of sterol synthesis in vivo defined in the organs of these two groups of rats, direct measurements were next made of the rates of LDL uptake by these same organs under identical in vivo conditions. The data in **Fig. 4** illustrate the rates of LDL clearance from the plasma per hr by 1 g of each major tissue. Two points warrants emphasis. First, in the two organs that are quantitatively most important in the rat for the degradation of rLDL, the liver and intestine (15), the rate of LDL uptake was essentially unaffected by aging. Thus, the liver from the young and old rats cleared rLDL at rates of 51 and 53 μl/hr per g, respectively, while the intestine from both groups of rats took up rLDL at the identical rate of 17 μl/hr per g. Second, aging did result in reductions in the mean uptake of rLDL by the adrenal gland (285 vs. 81 μl/hr per g) and by adipose tissue, skin and testis, all of which manifested essentially undetectable rates of LDL uptake in the older animals.

When these values were multiplied by the respective organ weights, the rates of rLDL clearance per hr by each organ were obtained, and these data are shown in **Fig. 5.** As is apparent, the values for the clearance of rLDL increased in nearly every organ with growth; these increases reflected primarily increases in organ size that

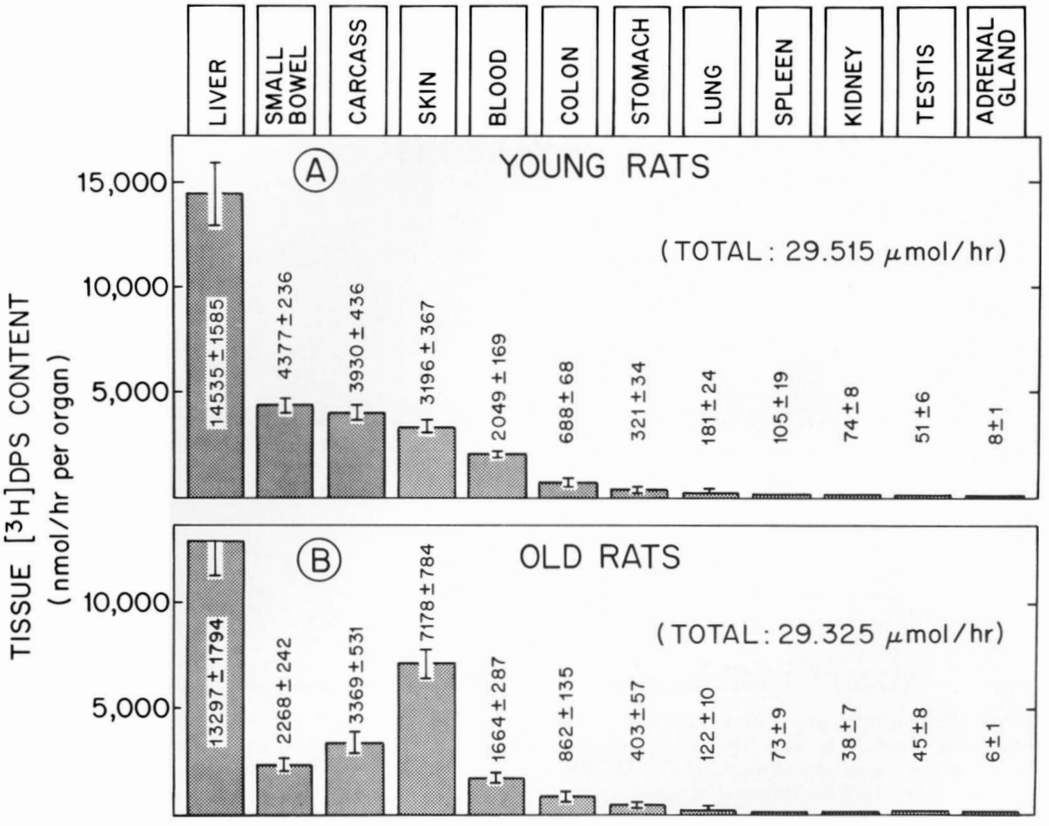

Fig. 3. Content of newly synthesized sterol in the various whole organs of young (panel A) and old (B) rats. These values equal the product of the tissue [^{3}H]DPS content (nmol/hr per g) shown in Fig. 2 and whole organ weight (Table 1). The mean absolute value of the total body [^{3}H]DPS content is also shown for each group of animals and represents the μmol of [^{3}H]water incorporated into DPS per hr.

were out of proportion to any changes that were observed in the rate of rLDL clearance per g of tissue (Fig. 4). However, the relative importance of each organ to rLDL degradation remained nearly constant with growth. For example, of the total rLDL clearance found in these ten organs, 50% and 62%, respectively, was detected in the liver of the young and old animals, while 24% was identified in the small bowel in both groups. Similarly, with the exception of skin, the percentage contribution of the remaining organs to rLDL degradation was small and essentially equal in the young and old rats.

DISCUSSION

These studies demonstrate that during the transition from the very active growth phase seen in young rats weighing only about 100 g to the more stable phase of less rapidly growing animals weighing about 450 g, the rate at which the body synthesizes cholesterol declines markedly by about 80% (when normalized to a constant body weight). In contrast, the rate of LDL uptake by the major organs remains essentially constant, as do the levels of both total and LDL-associated cholesterol in the plasma of these two groups of animals. This observation represents yet another example of the independent regulation of rates of tissue cholesterol synthesis and LDL uptake, and again emphasizes the primary role of regulating rates of cholesterol synthesis in order to meet changing demands for cellular cholesterol.

In the initial set of experiments it was established that the plasma cholesterol concentration and its distribution among the different lipoprotein density classes were similar (Fig. 1) in the growing and mature animals. This finding is consistent with previous reports that plasma cholesterol levels in the rat are stable during the first 6 months of life and, thereafter, increase only to a variable degree in different strains of rats (29–34). For example, the LDL-cholesterol concentration has been reported to be either increased (32, 33) or unchanged, even in senescent rats 22 months of age (34). The finding of similar concentrations of cholesterol in the major lipoprotein classes in the two groups of animals used in these studies is important since plasma lipoproteins may serve as one of the regulators of sterol synthesis (10) and, possibly, receptor-mediated LDL uptake in various extrahepatic

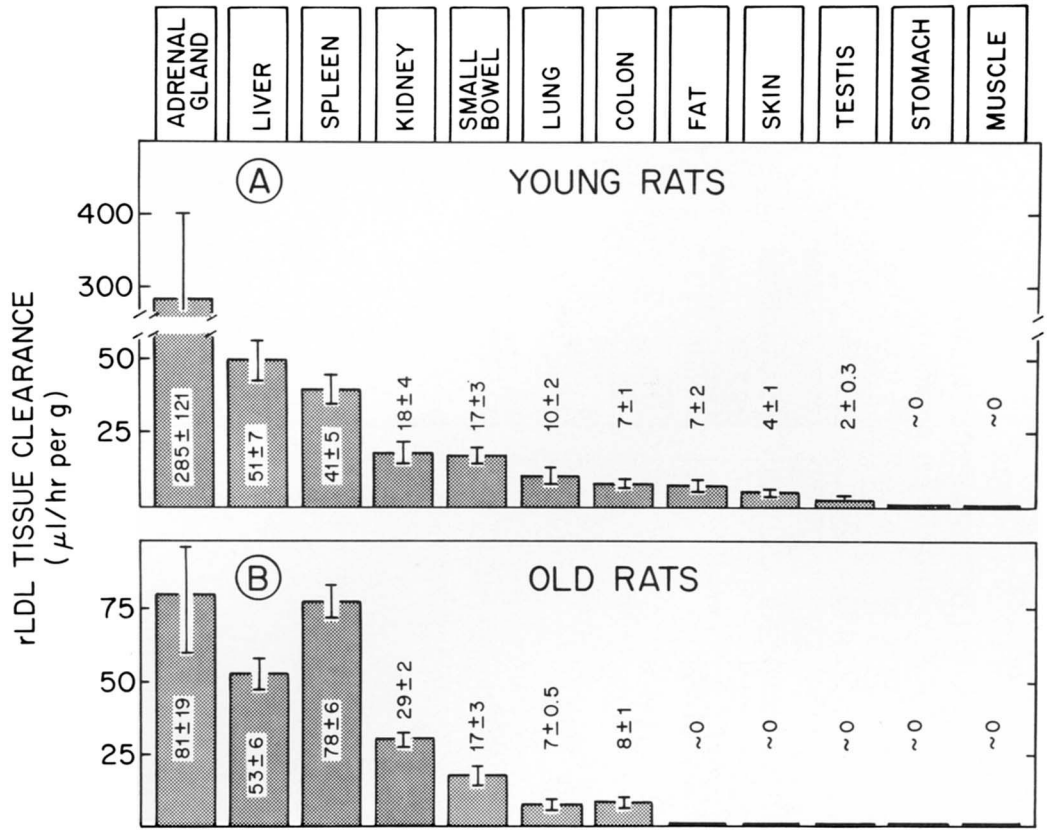

Fig. 4. Rates of clearance (uptake) of rLDL by the various tissues of young (panel A) and old (B) rats. The rats were fitted with femoral vein catheters and given a bolus of labeled homologous rat LDL followed by a constant infusion of the same lipoprotein to keep the plasma SA stable during the experiment. Groups of rats (n = 5) were killed at 2 and 5 hr and the tissues were processed as described in Materials and Methods. The rLDL tissue clearance (μl/hr per g) was calculated from the increase in the tissue space (μl/g) in each organ with time. Total body weight was 100 ± 2 g and 410 ± 5 g for the young and old rats, respectively. The data represent the slope ± 1 SEM of the best fit regression line.

tissues (17). Thus, the changes in rates of sterol synthesis observed in the various organs of the older rats cannot be attributed to a concomitant increase in plasma cholesterol levels but, rather, were probably a response to the diminished demand for cellular cholesterol as the rate of tissue growth diminished. This age-related decline in sterol synthesis rates has been described previously in tissues such as the liver (35, 36) and skin (37) using techniques based upon the measurement of the activity of 3-hydroxy-3-methylglutaryl-CoA reductase or the incorporation of [^{14}C]acetate into sterols as indicators of the rate of cholesterol synthesis. The [^{3}H]water technique used in the present investigation confirmed these observations in vivo and, in addition, further showed that the content of newly synthesized [^{3}H]DPS was lower in *every* organ of the mature animal when expressed per g of tissue.

However, in a broader context, the effect of aging on cholesterol synthesis in the whole animal must take into account not only the changes that occur in rates of sterol synthesis when expressed per g of tissue, but must also take into consideration changes that occur with respect to whole organ weight and with respect to the relative contribution of that organ to the whole-animal weight. The effects of growth on all three of these factors is taken into account when the absolute values of the [^{3}H]DPS content are calculated per organ per 100 g of whole-animal body weight, as has been done in **Table 2** (columns 1 and 2). As is apparent, in nearly all organs the rates of sterol synthesis are 3- to 9-fold higher in the young rats than in the older animals. Only in skin is this difference only 2-fold. Thus, whether the data are expressed per g of tissue or per whole organ (normalized to a constant body weight), the transition from rapid body growth to more stable body weights is accompanied by a marked decrease in the rate of sterol synthesis in all major organs.

These findings are in contrast to the effects of aging on rates of LDL uptake and degradation. In the liver and small bowel, the two organs that account for 60–65% of total LDL turnover in the rat (13), the rates of

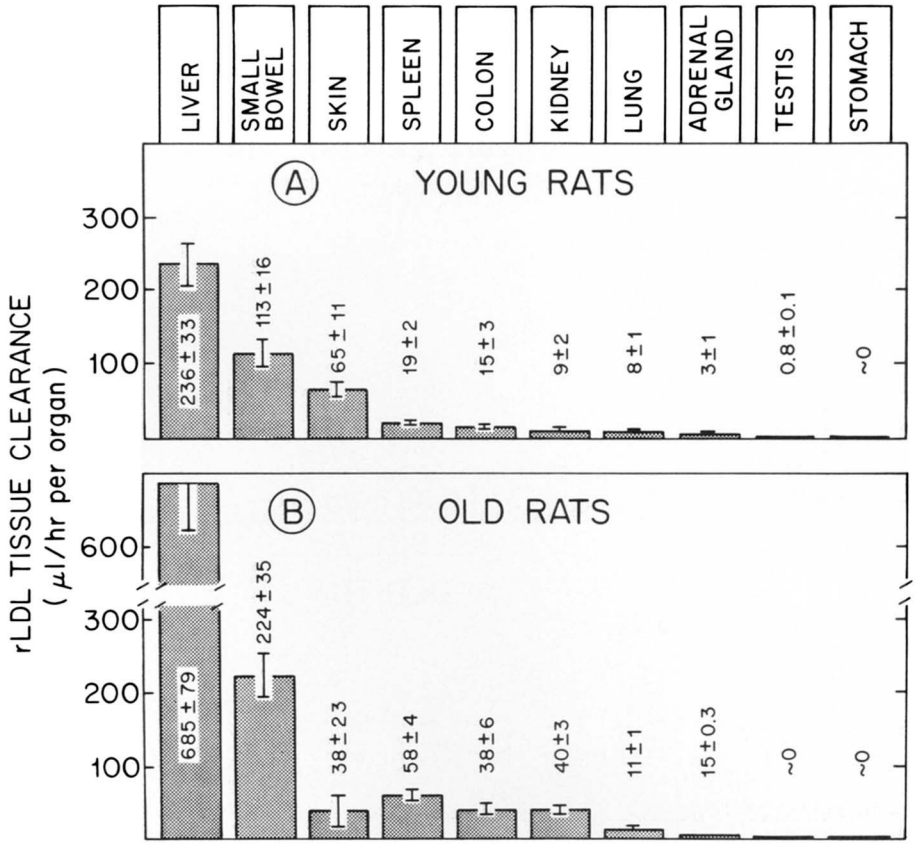

Fig. 5. Rates of clearance (uptake) of rLDL by the various whole organs of young (panel A) and old (B) rats. These values equal the product of the rLDL tissue clearance (μl/hr per g) shown in Fig. 4 and the whole organ weight (Table 1) of each tissue.

LDL uptake are identical in the young and old animals (Fig. 4). This is also true for other organs such as kidney, lung, and colon. Since >90% of this uptake is receptor-mediated (except in the intestine (16)), this finding indicates that the number of LDL receptors per g of tissue remains essentially constant during the transition from rapid to slow growth (13). Decreases in LDL uptake with aging were found only in the adrenal gland, adipose tissue, and skin, and none of these tissues is quantitatively very important in whole-animal LDL turnover (Fig. 5). When the rates of LDL clearance in each organ are normalized to a constant body weight (Table 2), the clearance of LDL by the liver decreased from 232 to 151 μl/hr per 100 g body weight and by the intestine from 111 to 49 μl/hr per 100 g body weight. These differences, it should be emphasized, were due to the fact that the liver and intestine represented 4.7% and 6.4%, respectively, of total body weight in the small rats but only 3.0% and 2.9%, respectively, in the large animals (Table 1). Hence, the older animals had a slightly lower rate of clearance of LDL from the plasma (normalized to 100 g body weight) than the smaller animals; this difference reflects the relatively smaller size of the liver and intestine in the large animals and not a change in the number of LDL receptors per g of tissue.

A slightly lower fractional catabolic rate for the clearance of ^{125}I-labeled LDL also has recently been reported

TABLE 2. Tissue [^{3}H]DPS content and LDL clearance in young and old rats normalized to a constant body weight of 100 g

	Tissue [^{3}H]DPS Content		Tissue rLDL Clearance	
	Young Rats	Old Rats	Young Rats	Old Rats
	nmol/hr per organ per 100 g body weight		*μl/hr per organ per 100 g body weight*	
A. Liver	14306	2935	232	151
B. Small bowel	4308	501	111	49
C. Carcass	3868	744		
D. Skin	3146	1585	64	8
E. Blood	2016	367		
F. Colon	677	190	15	8
G. Stomach	316	89	~0	~0
H. Lung	178	27	8	2
I. Spleen	103	16	19	13
J. Kidney	73	8	9	9
K. Testis	50	10	0.8	~0
L. Adrenal	8	1	3	0.3

The data in this table represent the whole organ [^{3}H]DPS content (Fig. 3) and rLDL clearance (Fig. 5) normalized to 100 g body weight.

in large rats (521 g, 0.055/hr) compared to smaller animals (252 g, 0.069/hr) (38). Unfortunately, these measurements were flawed by the fact that they were made using LDL of human origin which reacts very poorly with the rat LDL receptor (20, 39). When such measurements are made utilizing rLDL, the fractional catabolic rate actually equals approximately 0.21–0.24 (20, 40). However, taken together, these various data demonstrate that during the transition from a rapidly growing to more mature animal, the uptake of LDL by the various organs and the plasma LDL levels remain essentially constant under circumstances where the diminished needs for sterol by the various tissues is compensated for by profound decreases in the rates of cholesterol synthesis.

Finally, the two sets of data obtained in these studies provide the basis for quantitating the importance of local cholesterol synthesis and the uptake of cholesterol carried in LDL as sources for tissue sterol acquisition. The rates of [^{3}H]water incorporation into DPS can be converted to μg of cholesterol synthesized per hr in 1 g of each organ assuming that 1.45 μg atoms of carbon are incorporated into the cholesterol molecule per μg atom of ^{3}H that appears in [^{3}H]DPS (3, 26). Similarly, by multiplying the rates of LDL clearance in each organ by the concentration of LDL-cholesterol in 1 μl of plasma, the μg of cholesterol taken up per hr in 1 g of each tissue also can be obtained. Thus, as shown in **Fig. 6,** in the young animals the rates of tissue cholesterol acquisition from these two sources varied from approximately 100 μg/hr per g in the liver to only 1 to 2 μg/hr per g in muscle. Furthermore, in nearly all of the tissues, most of this sterol was acquired from local synthesis rather than from LDL uptake. The important exceptions to this are the adrenal gland and spleen where lipoprotein uptake was

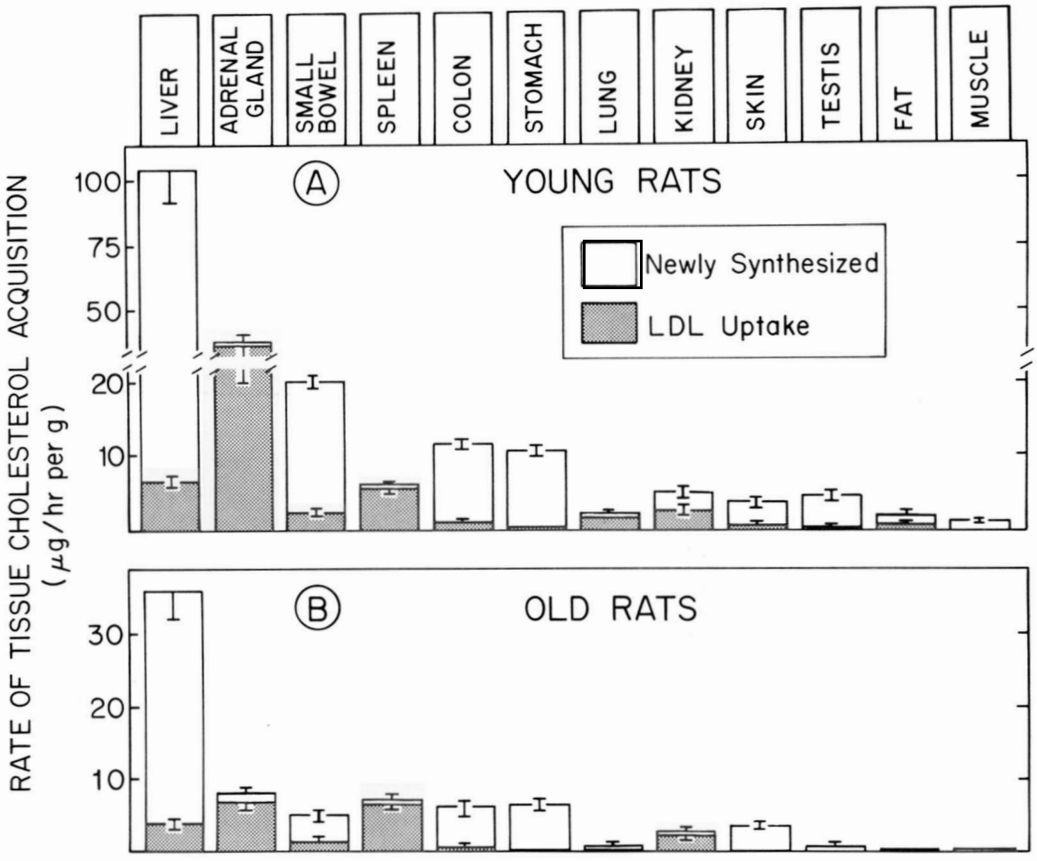

Fig. 6. Rates of tissue cholesterol acquisition from sterol synthesis and rLDL uptake in young (panel A) and old (B) rats. The values for cholesterol synthesis (μg/hr per g) were calculated from the absolute rates of [^{3}H]water incorporation into DPS (after correcting for uptake from blood (12)) using the factors 1.45 (C/^{3}H incorporation ratio) and 18 (the number of acetyl-CoA units incorporated per molecule of cholesterol) as detailed in reference 12. The data for rLDL-cholesterol uptake are based on the tissue clearances (μl/hr per g) as shown in Fig. 4 and the LDL cholesterol concentration in plasma of the respective age group. The open columns denote the amount of sterol derived from synthesis and the hatched bars denote the amount derived from rLDL uptake for the various organs. These calculations do not take into account the uptake of cholesterol from other lipoprotein sources such as high density lipoproteins (e.g., the adrenal glands) or chylomicrons (e.g., the liver).

quantitatively more important. With aging, the rates of tissue cholesterol acquisition decreased markedly (panel B) and this decrease was accomplished almost entirely by a decrease in the rate of cholesterol synthesis. It should be noted that these calculations do not take into account other sources for cholesterol in particular organs, e.g., the uptake of chylomicron-cholesterol by the liver (9) and of HDL-cholesterol by the adrenal gland (28).

Thus, these observations provide additional support for the concept that there is independent regulation of cholesterol synthesis and receptor-mediated LDL uptake in most tissues. Marked changes in cholesterol balance across the intestine (16), liver (15), or other tissue cells is accommodated for by appropriate reciprocal changes in rates of cholesterol synthesis while the rate of LDL uptake remains essentially unchanged. This accounts for the observation that in many species, including man, the plasma LDL-cholesterol levels remain remarkably constant under conditions where marked changes in cholesterol balance have been induced by dietary, surgical, or drug manipulations (41–43). Only under circumstances where adaptive responses of the biosynthetic pathway are exceeded (or blocked) are changes in tissue LDL uptake and, hence, levels of circulating LDL cholesterol, found (21, 22). These studies do not rule out the possibility that there may be important changes in LDL receptor activity in much older animals that may account for age-related increases in plasma LDL-cholesterol levels. Such changes, however, must be much smaller and not related directly to the much greater reductions in cholesterol synthetic activity that accompany such aging.

The authors wish to thank Leigh Riley, Edith Cole, Amy Nesbitt, and Rhonda El-Hage for their excellent technical assistance and Imogene Robison for her help in the preparation of the manuscript. These studies were supported by U.S. Public Health Service Research Grants HL-09610 and AM-19329, and by a grant from the Moss Heart Fund. During the period that these studies were carried out, Dr. Stange was supported by a grant from the Deutsche Forschungsgemeinschaft (STA 172/2-1).

Manuscript received 26 September 1983.

REFERENCES

1. Turley, S. D., and J. M. Dietschy. 1982. Cholesterol metabolism and excretion. *In* The Liver: Biology and Pathobiology. I. Arias, H. Popper, D. Schachter, and D. A. Shafritz, editors. Raven Press, New York. 467–492.
2. Westergaard, H., and J. M. Dietschy. 1976. The mechanism whereby bile acid micelles increase the rate of fatty acid and cholesterol uptake into the intestinal mucosal cell. *J. Clin. Invest.* **58:** 97–108.
3. Spady, D. K., and J. M. Dietschy. 1983. Sterol synthesis in vivo in 18 tissues of the squirrel monkey, guinea pig, rabbit, hamster, and rat. *J. Lipid Res.* **24:** 303–315.
4. Dietschy, J. M., and W. G. Gamel. 1971. Cholesterol synthesis in the intestine of man: regional differences and control mechanisms. *J. Clin. Invest.* **50:** 872–880.
5. Dietschy, J. M., and M. D. Siperstein. 1965. Cholesterol synthesis by the gastrointestinal tract: localization and mechanisms of control. *J. Clin. Invest.* **44:** 1311–1327.
6. Andersen, J. M., S. D. Turley, and J. M. Dietschy. 1982. Relative rates of sterol synthesis in the liver and various extrahepatic tissues of normal and cholesterol-fed rabbits. Relationship to plasma lipoprotein and tissue cholesterol levels. *Biochim. Biophys. Acta.* **711:** 421–430.
7. Stange, E. F., K. E. Suckling, and J. M. Dietschy. 1983. Synthesis and coenzyme A-dependent esterification of cholesterol in rat intestinal epithelium. Differences in cellular localization and mechanisms of regulation. *J. Biol. Chem.* **258:** 12868–12875.
8. Weis, H. J., and J. M. Dietschy. 1969. Failure of bile acids to control hepatic cholesterogenesis: evidence for endogenous cholesterol feedback. *J. Clin. Invest.* **48:** 2398–2408.
9. Nervi, F. O., H. J. Weis, and J. M. Dietschy. 1975. The kinetic characteristics of inhibition of hepatic cholesterogenesis by lipoproteins of intestinal origin. *J. Biol. Chem.* **250:** 4145–4151.
10. Andersen, J. M., and J. M. Dietschy. 1977. Regulation of sterol synthesis in 15 tissues of rat. II. Role of rat and human high and low density plasma lipoproteins and of rat chylomicron remnants. *J. Biol. Chem.* **252:** 3652–3659.
11. Balasubramaniam, S., J. L. Goldstein, J. R. Faust, and M. S. Brown. 1976. Evidence for regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and cholesterol synthesis in nonhepatic tissues of rat. *Proc. Natl. Acad. Sci. USA.* **73:** 2564–2568.
12. Turley, S. D., J. M. Andersen, and J. M. Dietschy. 1981. Rates of sterol synthesis and uptake in the major organs of the rat in vivo. *J. Lipid Res.* **22:** 551–569.
13. Dietschy, J. M., D. K. Spady, and E. F. Stange. 1983. Quantitative importance of different organs for cholesterol synthesis and low density lipoprotein degradation. *Biochem. Soc. Trans.* **11:** 639–641.
14. Spady, D. K., D. W. Bilheimer, and J. M. Deitschy. 1983. Rates of receptor-dependent and -independent low density lipoprotein uptake in the hamster. *Proc. Natl. Acad. Sci. USA.* **80:** 3499–3503.
15. Dietschy, J. M., S. D. Turley, and D. K. Spady. 1983. The role of the liver in lipid and lipoprotein metabolism. In Liver in Metabolic Diseases. L. Bianchi, W. Gerok, L. Landmann, K. Sickinger, and G. A. Stalder, editors. MTP Press Limited, Boston, The Hague, Dordrecht, Lancaster.
16. Stange, E. F., and J. M. Dietschy. 1983. Cholesterol synthesis and low density lipoprotein uptake are regulated independently in rat small intestinal epithelium. *Proc. Natl. Acad. Sci. USA.* **80:** 5739–5743.
17. Goldstein, J. L., and M. S. Brown. 1977. The low-density lipoprotein pathway and its relation to atherosclerosis. *Annu. Rev. Biochem.* **46:** 897–930.
18. Brown, M. S., and J. L. Goldstein. 1979. Receptor-mediated endocytosis: insights from the lipoprotein receptor system. *Proc. Natl. Acad. Sci. USA.* **76:** 3330–3337.
19. Pangburn, S. H., R. S. Newton, C-M. Chang, D. B. Weinstein, and D. Steinberg. 1981. Receptor-mediated catabolism of homologous low density lipoproteins in cultured pig hepatocytes. *J. Biol. Chem.* **256:** 3340–3347.
20. Koelz, H. R., B. C. Sherrill, S. D. Turley, and J. M. Dietschy. 1982. Correlation of low and high density lipoprotein binding in vivo with rates of lipoprotein degradation in the rat.

A comparison of lipoproteins of rat and human origin. *J. Biol. Chem.* **257:** 8061–8072.
21. Kovanen, P. T., D. W. Bilheimer, J. L. Goldstein, J. J. Haramillo, and M. S. Brown. 1981. Regulatory role for hepatic low density lipoprotein receptors in vivo in the dog. *Proc. Natl. Acad. Sci. USA.* **78:** 1194–1198.
22. Bilhemier, D. W., S. M. Grundy, M. S. Brown, and J. L. Goldstein. 1983. Mevinolin and colestipol stimulate receptor-mediated clearance of low density lipoprotein from plasma in familial hypercholesterolemia heterozygotes. *Proc. Natl. Acad. Sci. USA.* **80:** 4124–4128.
23. Jeske, D. J., and J. M. Dietschy. 1980. Regulation of rates of cholesterol synthesis in vivo in the liver and carcass of the rat measured using [^{3}H]water. *J. Lipid Res.* **21:** 364–376.
24. Pittman, R. C., A. D. Attie, T. E. Carew, and D. Steinberg. 1979. Tissue sites of degradation of low density lipoprotein: application of a method for determining the fate of plasma proteins. *Proc. Natl. Acad. Sci. USA.* **76:** 5345–5349.
25. Spady, D. K., S. D. Turley, and J. M. Dietschy. 1983. Dissociation of hepatic cholesterol synthesis from hepatic low-density lipoprotein uptake and biliary cholesterol saturation in female and male hamsters of different ages. *Biochim. Biophys. Acta.* **753:** 381–392.
26. Andersen, J. M., and J. M. Dietschy. 1979. Absolute rates of cholesterol synthesis in extrahepatic tissues measured with ^{3}H-labeled water and ^{14}C-labeled substrates. *J. Lipid Res.* **20:** 740–752.
27. Munford, R. S., J. M. Andersen, and J. M. Dietschy. 1981. Sites of tissue binding and uptake in vivo of bacterial lipopolysaccharide-high density lipoprotein complexes. Studies in the rat and squirrel monkey. *J. Clin. Invest.* **68:** 1503–1513.
28. Andersen, J. M., and J. M. Dietschy. 1981. Kinetic parameters of the lipoprotein transport systems in the adrenal gland of the rat determined in vivo. Comparison of low and high density lipoproteins of human and rat origin. *J. Biol. Chem.* **256:** 7362–7370.
29. Carlson, L. A., S. O. Froberg, and E. R. Nye. 1968. Effect of age on blood and tissue lipid levels in the male rat. *Gerontologia.* **14:** 65–79.
30. Yamamoto, M., and Y. Yamamura. 1971. Changes of cholesterol metabolism in the ageing rat. *Atherosclerosis.* **13:** 365–374.
31. Story, J. A., S. A. Tepper, and D. Kritchevsky. 1976. Age-related changes in the lipid metabolism of Fisher 344 rats. *Lipids.* **11:** 623–627.
32. Uchida, K., Y. Nomura, M. Kadowaki, H. Takase, K. Takano, and N. Takeuchi. 1978. Age-related changes in cholesterol and bile acid metabolism in rats. *J. Lipid Res.* **19:** 544–552.
33. Liepa, G. U., E. J. Masoro, H. A. Bertrand, and B. P. Yu. 1980. Food restriction as a modulator of age-related changes in serum lipids. *Am. J. Physiol.* **238:** E253–E257.
34. Van Lenten, B. J., and P. S. Roheim. 1982. Changes in the concentrations and distributions of apolipoproteins of the aging rat. *J. Lipid Res.* **23:** 1187–1195.
35. Shefer, S., S. Hauser, V. Lapar, and E. H. Mosbach. 1972. HMG CoA reductase of intestinal mucosa and liver of the rat. *J. Lipid Res.* **13:** 402–412.
36. Takeuchi, N., Y. Yamamura, Y. Katayama, C. Hayashi, and K. Uchida. 1976. Impairment of feed back control and induction of cholesterol synthesis in rat by aging. *Exp. Gerontol.* **11:** 121–126.
37. Trout, E. C., K. Y. T. Kao, C. A. Hizer, and T. H. McGavack. 1962. In vitro synthesis of free and ester cholesterol in various tissues of young and old rats. *J. Gerontol.* **17:** 363–368.
38. Sykes, M., W. M. Cnoop-Koopmans, P. Julien, and A. Angel. 1981. The effects of hypothyroidism, age, and nutrition on LDL catabolism in the rat. *Metabolism.* **30:** 733–738.
39. Innerarity, T. L., R. E. Pitas, and R. W. Mahley. 1980. Disparities in the interaction of rat and human lipoproteins with cultured rat fibroblasts and smooth muscle cells. Requirements for homology for receptor binding activity. *J. Biol. Chem.* **255:** 11163–11172.
40. Mahley, R. W., K. H. Weisgraber, G. W. Melchior, T. L. Innerarity, and K. S. Holcombe. 1980. Inhibition of receptor-mediated clearance of lysine and arginine-modified lipoproteins from the plasma of rats and monkeys. *Proc. Natl. Acad. Sci. USA.* **77:** 225–229.
41. Weis, H. J., and J. M. Dietschy. 1974. Adaptive responses in hepatic and intestinal cholesterogenesis following ileal resection in the rat. *Eur. J. Clin. Invest.* **4:** 33–41.
42. Ginsberg, H., N. A. Le, C. Mays, J. Gibson, and W. V. Brown. 1981. Lipoprotein metabolism in nonresponders to increased dietary cholesterol. *Arteriosclerosis.* **1:** 463–470.
43. Kesaniemi, Y. A., and S. M. Grundy. 1984. Turnover of low density lipoproteins during inhibition of cholesterol absorption. *Arteriosclerosis.* **4:** 41–48.