Cholesterol synthesis in vivo and in vitro in the WHHL rabbit, an animal with defective low density lipoprotein receptors

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Abstract These studies were undertaken to measure rates of synthesis of digitonin-precipitable sterols in vivo and in vitro in control rabbits (New Zealand (NZ) control) and in homozygous Watanabe heritable hyperlipidemic rabbits (WHHL) that lack receptors for low density lipoproteins (LDL). The plasma cholesterol concentration in NZ control fetuses equaled 79 mg/dl, rose to 315 mg/dl 12 days after birth, and fell to 80 mg/dl in young adult animals. At these same ages, cholesterol concentrations in the WHHL animals equal 315, 625, and 715 mg/dl, respectively. The rate of whole animal sterol synthesis in vivo, expressed as the μmol of [^{3}H]water incorporated into sterols per hr per kg of body weight, was lower in the WHHL animals than in the NZ controls both in the fetuses (108 vs 176) and in the adult animals (48 vs 66). In adult NZ controls the content of newly synthesized sterols (rate of sterol synthesis) per g of tissue was highest in the liver (538 nmol/g per hr), adrenal gland (438), small bowel (371), and ovary (225) while lower rates of synthesis were found in 15 other tissues. In the WHHL rabbits a higher content of [^{3}H]sterols was found only in the adrenal gland (2,215) while synthesis was suppressed in the liver (310), colon, lung, and kidney, and was unchanged in the remaining organs. These findings were confirmed by measurements of rates of sterol synthesis in the same tissues in vitro. When whole organ weight was taken into consideration, the tissues that were the major contributors to whole body sterol synthesis in both types of rabbits were liver, small bowel, skin, and carcass. However, it was the lower rate of synthesis in the liver of the WHHL animals that alone accounted for the lower rate of whole animal sterol synthesis seen in these rabbits. These studies demonstrate that in WHHL animals that lack LDL receptors and that have very high levels of circulating LDL cholesterol, the rate of cholesterol synthesis in nearly all tissues is normal but in the liver is significantly suppressed. Only the adrenal gland manifested enhanced synthesis. Such findings suggest that in the WHHL rabbit where LDL receptor activity is reduced and plasma LDL levels rise, mechanisms other than receptor-mediated LDL uptake may act to deliver cholesterol to the cells of the various organs and to the liver.—**Dietschy, J. M., T. Kita, K. E. Suckling, J. L. Goldstein, and M. S. Brown.** Cholesterol synthesis in vivo and in vitro in the WHHL rabbit, an animal with defective low density lipoprotein receptors. *J. Lipid Res.* 1983. **24:** 469–480.

Supplementary key words liver • small intestine • adrenal glands • [^{3}H]water • LDL receptors

Homozygous Watanabe heritable hyperlipidemic (WHHL) rabbits have a 10-fold elevation in the plasma cholesterol level that results from a defect in the gene for the low density lipoprotein (LDL) receptor (1–3). The genetic defect in the WHHL rabbit is analogous to the defect in the LDL receptor gene in humans with familial hypercholesterolemia (FH) (4). The LDL receptor normally mediates the uptake and degradation of LDL and intermediate density lipoproteins (IDL) by body cells. When the receptor is absent, as in homozygous WHHL rabbits and FH homozygotes, plasma LDL and IDL are not degraded with normal effficiency and the lipoproteins accumulate in high levels in plasma, and atherosclerosis may occur (5).

Lipoproteins that bind to cells via the LDL receptor enter the cell by receptor-mediated endocytosis and undergo degradation, releasing their cholesterol for use in cellular metabolism. In tissue culture, cells have been shown to use the cholesterol derived from LDL under two conditions: *1*) when the cells are rapidly dividing and hence require cholesterol for new membrane synthesis; and *2*) when steroid-secreting cells, such as cultured adrenal cells, require cholesterol for steroid synthesis (6). If LDL is absent or if the receptor is nonfunctional and cells have a demand for cholesterol for one of the above processes, they satisfy this demand by increasing their cholesterol synthesis rate. This increase in cholesterol synthesis is mediated by an increase in the activity of the rate-controlling enzyme 3-hydroxy-3-

Abbreviations: WHHL, homozygous Watanabe heritable hyperlipidemic; LDL, low density lipoproteins; FH, familial hypercholesterolemia; IDL, intermediate density lipoproteins; HMG, 3-hydroxy-3-methylglutaryl; NZ, New Zealand; DPS, digitonin-precipitable sterols; ACAT, acyl-CoA:cholesterol acyltransferase; SA, specific activity.

methylglutaryl coenzyme A reductase (HMG CoA reductase). As a result of their receptor deficiency, cultured fibroblasts from FH homozygotes have an increased rate of cholesterol synthesis when they are growing rapidly. However, when FH homozygote cells, as well as normal cells, become confluent, they no longer require large amounts of cholesterol for growth. Under these conditions the cholesterol synthesis rate declines even in cells that have no LDL receptors (7).

More recently it has also been shown that chemical modification of the LDL particle blocks its interaction with the LDL receptor on the fibroblast (8, 9). Furthermore, modification such as reductive methylation has also been found to block specific LDL binding to organs such as the liver and adrenal gland under in vivo conditions and this reduced binding, in turn, is associated with a much slower rate of degradation of the circulating LDL (10). Using this technique, attempts have been made to define the quantitative importance of such "receptor-independent" LDL degradation in the whole animal by measuring the plasma turnover or clearance rate of normal and chemically modified homologous LDL in several animal species. Such studies suggest that in the monkey, rabbit, and rat approximately 50%, 33%, and 43%, respectively, of plasma LDL is cleared by such a non-receptor-mediated mechanism (9–11).

The quantitative importance of receptor-dependent and receptor-independent LDL uptake in specific differentiated tissues has not yet been determined although it is very likely that at low or physiological plasma LDL concentrations, receptor-mediated uptake is of paramount importance in most tissues. However, since receptor-independent LDL transport appears to take place by a nonsaturable process (10), it is likely that at higher plasma LDL concentrations (or in the absence of LDL receptors) this transport mechanism becomes physiologically much more important. In theory, then, high circulating levels of LDL could suppress cholesterol synthesis rates even in tissues that manifest little receptor-mediated LDL uptake.

One approach to better understanding the interrelationships of these processes in the tissues of the intact animal would be to measure rates of cholesterol synthesis in the various organs of the WHHL rabbit, which lacks nearly all LDL receptor activity and which has high circulating levels of LDL (1, 2). Thus, in the present experiments, rates of sterol synthesis were measured in control animals and in WHHL rabbits that varied in age from fetuses to young adults. The results demonstrate that all tissues of the WHHL animal, except the adrenal gland, showed normal or somewhat suppressed rates of cholesterol synthesis at all ages.

MATERIALS AND METHODS

Experimental animals

The female control rabbits (NZ control rabbits) used in these studies were of the New Zealand White strain (Hickory Hill Rabbitry, Flint, TX). The Watanabe heritable hyperlipidemic rabbits were raised in Dallas from a breeding pair originally supplied by Watanabe (1, 12, 13). For the purposes of these studies, only female NZ control and female homozygous WHHL animals were used. Some experiments also were carried out using fetuses (removed from the mother 1 day prior to the expected date of birth) or suckling infants (killed 4 and 12 days after birth) from both NZ control and WHHL mothers. All rabbits used in these studies were maintained in individual cages under circumstances where there was regular cycling of the light (approximately 14 hr of light and 10 hr of darkness) and free access to water and Wayne Rabbit Ration (Allied Mills, Inc., Chicago, IL) for at least 2 weeks before use. By direct analysis (gas–liquid chromatography) this diet contained 0.01 mg/g of cholesterol (14). All animals were weighed regularly during the 2-week period and any rabbit that did not gain weight at rates equal to its cohorts was excluded from the study.

Determination of sterol synthesis in vivo

As described in detail elsewhere (15) at between 0900 and 1100 hr the rabbits were administered 500 mCi of [^{3}H]water intravenously through a lateral ear vein and then placed in a well-ventilated fume hood. They received no further fluid or food and were killed exactly 1 hr later using an intravenous injection of pentobarbital. The abdomen was immediately opened and an exact aliquot of blood was aspirated from the abdominal aorta. Various organs were then removed, rinsed in cold isotonic saline to remove excess blood, weighed, and cut into slices 2 to 3-mm thick with a razor blade. Three aliquots of these slices from each organ were again rinsed in saline, blotted dry, weighed on an electronic recording balance, and placed in glass tubes for saponification. For the larger organs the aliquots weighed 500 to 800 mg, whereas for the smaller organs, e.g., adrenal gland and ovary, the whole tissue was used. The following tissues were sampled: liver, adrenal gland, ovary, stomach, spleen, lung, colon, whole blood, pancreas, brain (cerebrum), kidney, heart, adipose tissue (retroperitoneal fat), muscle (medial thigh muscle), fallopian tubes, and aorta. The entire small intestine was removed and saponified. After removal of these organs the animal was skinned and this entire tissue (hair, skin, and subcutaneous fat) was weighed and saponified. Finally, the entire remaining carcass was placed in a large vessel

and saponified. A portion of the whole blood sample was centrifuged to obtain plasma in which to measure the level of cholesterol and the specific activity (SA) of plasma water. Since no tissue was discarded, "whole animal" sterol synthesis rates could be calculated as the sum of the contents of newly synthesized sterol in the individual organs (15).

In those experiments utilizing fetuses, the [^{3}H]water was administered intravenously to the mother and the fetuses were removed from the uterus 1 hr later for analysis. It was demonstrated that the SA of the plasma water of the fetus rapidly equilibrated with the SA of the plasma water of the mother. In these studies and in those using the 4- and 12-day-old suckling infants, the content of newly synthesized sterol was measured in only four tissue compartments: these included the liver, the entire gastrointestinal tract (stomach, small and large intestine), brain, and all remaining tissues in the carcass. Each of these tissues was saponified in toto so that "whole animal" sterol synthesis rates equaled the sum of the content of newly synthesized sterols present in the four tissue fractions. Samples of blood were taken as well from the aorta or by cardiac puncture to measure the level of plasma cholesterol and the SA of plasma water in each animal. Finally, blood samples also were obtained from each donor mother so that the SA of the maternal plasma water could be directly compared to the SA of the plasma water found in each of the fetuses.

Determination of sterol synthesis in vitro

Rates of sterol synthesis also were measured in vitro in the following tissues: liver, adrenal gland, ovary, small bowel, kidney, spleen, lung, skeletal muscle, brain, stomach, skin, fallopian tubes, pancreas, aorta, and adipose tissue. In these studies the animals were killed and the tissues were immediately removed and chilled. The entire small intestine was removed and divided into ten equal segments, numbered sequentially from 1 to 10 beginning with the most proximal segment of duodenum between the gastroduodenal junction and the entrance of the common bile duct into the intestine. These different tissues were then sliced as previously described and incubated in 5 ml of Krebs' bicarbonate buffer containing 4 μCi of [1-^{14}C]octanoate (New England Nuclear, Boston, MA) at a concentration of 1.0 mM sodium octanoate. The incubations were carried out in a metabolic shaker at 100 oscillations per min for 90 min at 37°C (16, 17).

Analytic procedures

As previously described, the aliquots of tissue obtained from the in vivo studies (sterols labeled with ^{3}H) or from the in vitro incubations (sterols labeled with ^{14}C) were saponified with alcoholic KOH. The sterols were then extracted quantitatively and precipitated as the digitonides. These digitonin-precipitable sterols (DPS) were dried under vacuum and the digitonides were split with pyridine. The free sterols were extracted with diethyl ether, dried under vacuum, and assayed for ^{3}H or ^{14}C content (18, 19). In the in vitro experiments in which [1-^{14}C]octanoate was used as the labeled precursor, rates of incorporation of [1-^{14}C]octanoate into CO_2 were also determined (16, 17). The tissue and microsomal content of unesterified and esterified cholesterol was determined utilizing silicic acid/celite columns as previously described (20). The activity of acyl-CoA:cholesterol acyltransferase (ACAT) was assayed in washed microsomes of rabbit livers essentially as described previously for the rat (21). One mg of microsomal protein was preincubated with 3.3 mM glutathione and 0.5 mg of defatted bovine serum albumin in 0.5 ml of 0.1 M potassium phosphate buffer (pH 7.4) at 37°C for 3 min. [1-^{14}C]Oleoyl-CoA (40 nmol diluted to a specific activity of 1.25–2.50 μCi/μmol) was added and the reaction was allowed to proceed for an additional 5 min. The incubation was stopped by the addition of 1 ml of methanol and the radioactive product was isolated by thin-layer chromatography and counted as described previously (21). Under the conditions of this assay the reaction was linear with respect to time for up to 10 min and with respect to microsomal protein up to 2 mg. Plasma cholesterol levels were measured as previously described (22).

Calculations

In the in vivo experiments it was necessary to determine the mean SA of body water throughout the 1-hr interval over which rates of sterol synthesis were being measured. As previously described (18) this value, expressed as cpm of ^{3}H/nmol of water, was calculated by the following equation:

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

The term 1.09 corrects the SA of plasma water determined at 1 hr after injection of the [^{3}H]water to the mean SA of body water present throughout the 1-hr period of time.

Rates of sterol synthesis (or newly synthesized sterol content) in each tissue in vivo were expressed as the nmol of [^{3}H]water incorporated into DPS per hr per g of tissue (nmol/hr per g) and were calculated using the following relationship:

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

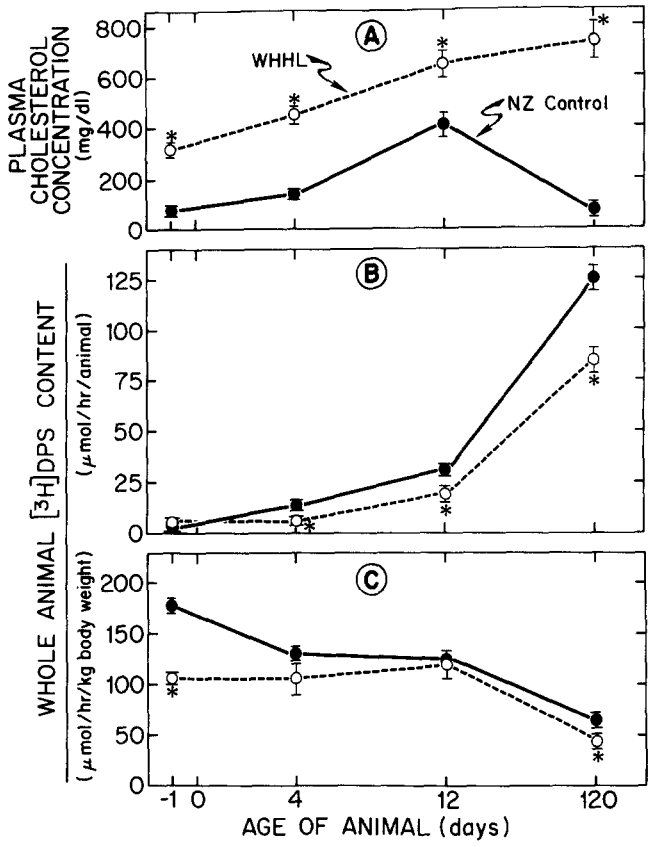

Fig. 1. Plasma cholesterol levels and rates of sterol synthesis in vivo in animals of varying age. Both the NZ control and WHHL female rabbits were maintained on the same diets under conditions of light cycling, as described in the Materials and Methods section. Rates of incorporation of [^{3}H]water into DPS in vivo over a 1-hr period were then measured in whole animals of different ages. These rates of whole animal synthesis are expressed in two ways, i.e., as the μmol of [^{3}H]water incorporated into DPS per hr per animal (panel B) and per kg of body weight (panel C). The horizontal axis shows the age of the animals where zero equals the time of birth. Four groups of rabbits were studied: these included fetuses taken from the mother 1 day prior to the anticipated date of birth (−1 day), newborn animals that were nursing (4 and 12 days after birth), and adult animals that were approximately 120 days old. The data represent mean values ± 1 SEM for results obtained in four to six animals in each group. The asterisks represent those values found in the WHHL rabbits that were significantly different from the values found in the NZ control animals.

These rates (or contents) were also presented as the μmol of [^{3}H]water incorporated into DPS per hr per whole organ (μmol/hr per organ). Whole animal sterol synthesis rates were calculated as the sums of the contents of [^{3}H]DPS in all of the organs (19).

In those experiments in which sterol synthesis was measured in vitro, the rates were calculated as the nmol of acetyl CoA units, i.e., C_2 units incorporated into DPS per g per hr using the following relationship (23):

$$\frac{(\text{cpm } [^{14}\text{C}]\text{DPS})(6)}{(1.5 \text{ hr})(\text{g tissue wt})(\text{SA } [1\text{-}^{14}\text{C}]\text{octanoate})}.$$

The factor of 6 converts nmol of octanoate to nmol of C_2 units and, at the same time, corrects for loss of 33% of the ^{14}C as $^{14}CO_2$ during conversion of [1-^{14}C]acetyl CoA to sterols. These rates correspond to the normalized C_2 fluxes into DPS, as previously defined (23), and do not include a correction for dilution of the SA of the intracellular acetyl CoA pool. Hence, they are useful in comparing relative rates of sterol synthesis in the same organ in the two types of rabbits, but should not be used as a measure of absolute rates of cholesterol synthesis in any tissue.

In all experiments, the data are presented as mean values ± 1 SEM. The significance of a difference in the values obtained with the WHHL rabbits relative to the NZ controls was tested at the $P < 0.05$ level and, if significant, is indicated in the figures and table by an asterisk.

RESULTS

Effect of aging on whole animal sterol synthesis

The initial group of studies was undertaken to determine how the rate of sterol synthesis in the whole animal varied at different ages in the NZ control and WHHL animals. Four different age groups were studied: these included fetuses taken from the uterus 1 day before the expected date of birth, suckling infants at 4 and 12 days after birth, and young adult animals approximately 120 days old. As illustrated in panel A of **Fig. 1,** the plasma cholesterol concentration in the NZ control fetuses averaged only 79 mg/dl while the fetuses from the homozygous WHHL mothers already manifested marked hypercholesterolemia with an average plasma cholesterol level of 315 mg/dl. When the newborn infants began to nurse, plasma cholesterol levels increased significantly in the NZ control rabbits to a mean of 137 mg/dl and 408 mg/dl at 4 and 12 days, respectively. Nevertheless, the levels of the suckling WHHL rabbits increased to even higher levels reaching average values of 440 mg/dl and 625 mg/dl, respectively, in these two age groups. Once weaned and placed on a low cholesterol rabbit chow, plasma sterol levels fell precipitously to 80 mg/dl in the adult control animals but remained high at 715 mg/dl in the WHHL rabbits.

In the face of this large difference in the plasma cholesterol concentration, the absolute rate of whole animal sterol synthesis in the fetuses was the same and equaled 4.2 μmol/hr in both the NZ control and WHHL animals (panel B). However, the weight of the WHHL fetuses was nearly twice as great as the NZ control fetuses so that when whole animal synthesis rates were normalized to a constant body weight, the rate of sterol synthesis in the WHHL fetuses was significantly lower (108

μmol/hr per kg body weight) than that of the NZ control fetus (176 μmol/hr per kg body weight) (panel C). In the other three age groups, the absolute rates of sterol synthesis in the WHHL animals was consistently suppressed below the rates seen in the comparably aged control animals (panel B). Once again, however, there were significant differences in animal weights in that the 4- and 12-day-old suckling animals from the WHHL mothers were much lighter than the comparable control animals. Hence, when expressed per kg body weight (panel C) sterol synthesis in the two groups of suckling animals was similar while the adult WHHL rabbits, like the fetuses, manifested a rate of synthesis that was significantly lower than that seen in the NZ control animals. It should be emphasized that in all age groups studied, and regardless of how the data were expressed, the WHHL animals never manifested rates of sterol synthesis higher than the control animals and, in most cases, were actually synthesizing sterols at less than control rates.

In order to define the reasons for these differences, content of newly synthesized sterol was measured in four different tissue compartments in these same groups of animals. As seen in **Fig. 2,** the content of [^{3}H]DPS per g of liver was the same in the NZ control and WHHL fetuses (panel A). However, with the onset of nursing and the consequent high intake of cholesterol-rich milk, the rate of sterol synthesis in the liver of both groups was markedly suppressed. Hepatic sterol synthesis returned to a relatively high rate in the adult NZ control animals but remained partially suppressed in WHHL rabbits. In direct contrast to these findings in the liver, sterol synthesis in the gastrointestinal tract (panel B) and remaining tissues of the carcass (panel D) was significantly lower in the fetuses of the WHHL rabbits than in the same tissues of the control animals, but these differences completely disappeared by the time the animals reached 120 days of age. The rate of sterol synthesis in the brain (panel C) progressively declined as the animals aged and was the same in the two groups of rabbits. Thus, it is apparent from these data that the lower rates of whole animal sterol synthesis seen in the WHHL fetuses (panel C, Fig. 1) were due primarily to much lower rates of sterol synthesis in the extrahepatic tissues of these animals (Fig. 2) while the lower rates of synthesis manifested in the adult WHHL animals was due primarily to a significantly lower rate of hepatic cholesterol synthesis.

Sterol synthesis in vivo in the adult animal

In order to delineate differences that might exist in rates of sterol synthesis in the other major tissues, detailed studies were next undertaken to measure rates of synthesis in 19 tissues of adult animals under in vivo

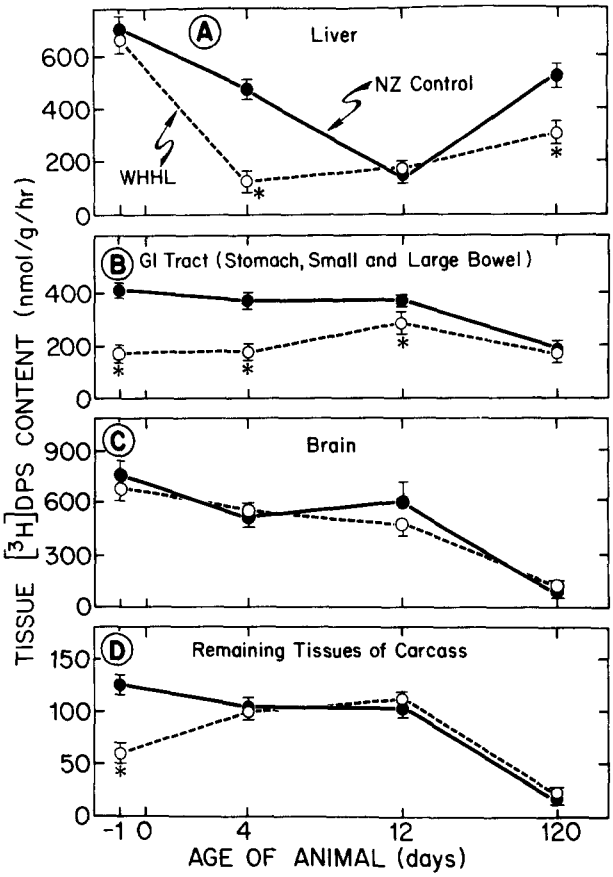

Fig. 2. Content of newly synthesized sterol in four major tissue compartments of rabbits of different ages. These data were derived from the same animals described in the legend to Fig. 1 and represent the nmol of [^{3}H]water incorporated into DPS by 1 g of liver (panel A), the stomach and small and large bowel combined (B), brain (C), and all remaining tissues of the carcass combined (D). The data represent mean values ± 1 SEM for results obtained in four to six animals.

conditions. These animals were maintained on a low cholesterol diet under conditions of light cycling, as described in the Materials and Methods section, for at least 2 weeks before being used in these studies. The animals were approximately 3 months of age and were matched closely with respect to weights: the NZ control and WHHL animals weighed 1860 ± 109 g and 1794 ± 126 g, respectively.

The data in **Fig. 3** show the content of newly synthesized sterol per g of tissue in 19 organs of the control and WHHL animals. As has been described before (15) in control NZ rabbits, the highest contents of [^{3}H]DPS were found in the liver, small intestine, adrenal gland, and ovaries (panel A). All other organs had at least some newly synthesized sterol, but the amounts were much lower, particularly in the carcass (predominantly skeletal muscle, bone, and marrow), cardiac muscle, skeletal muscle, and fat. The WHHL rabbits showed a nearly identical profile of [^{3}H]DPS contents (panel B) with two important exceptions. First, the content of newly syn-

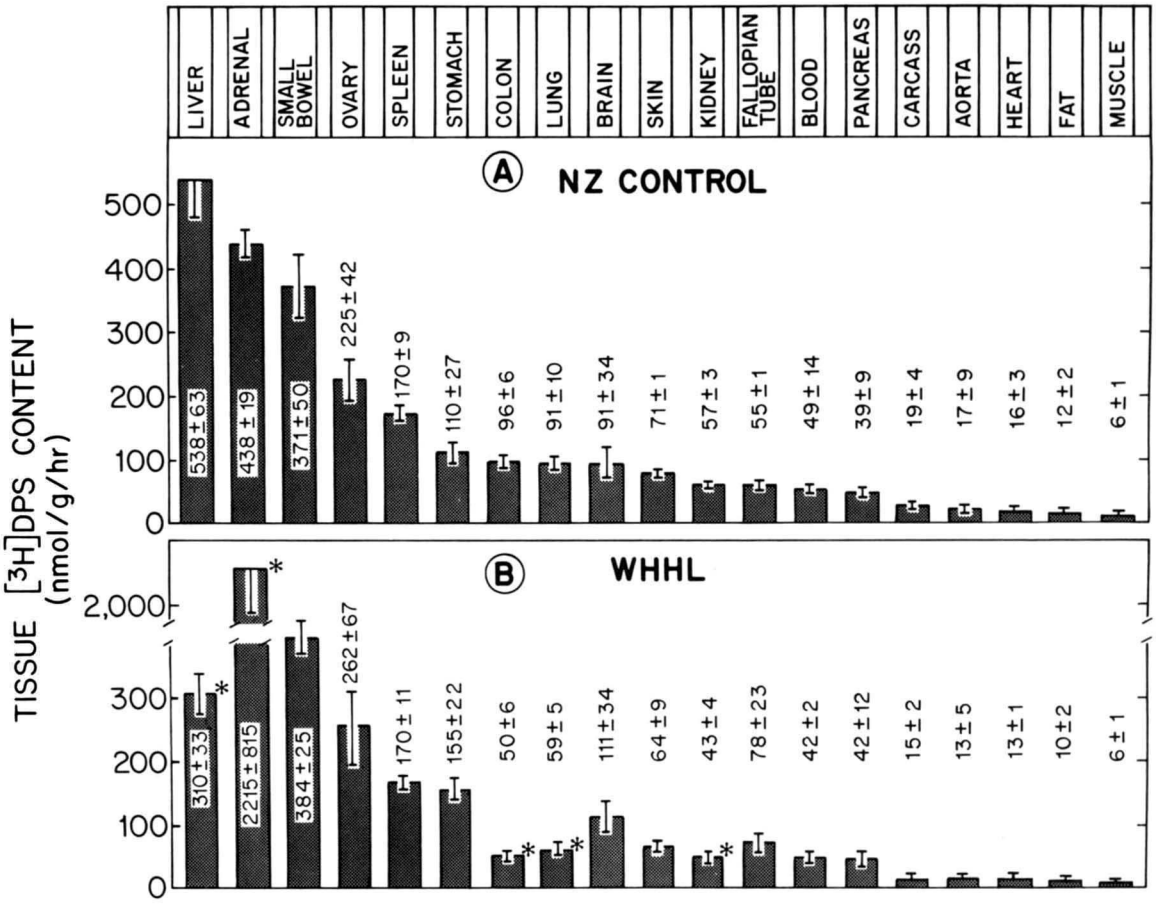

Fig. 3. The content of sterols newly synthesized in vivo per g of tissue in 19 organs of adult female rabbits. These animals were of similar age and were matched with respect to weight. The NZ control and WHHL animals weighed 1860 ± 109 g and 1794 ± 126 g, respectively. After being maintained on identical diets under conditions of light cycling for at least 2 weeks, each animal was administered [^{3}H]water intravenously and killed 1 hr later. The nmol of [^{3}H]water that were incorporated into DPS per g of tissue per hr under these in vivo conditions is given for each organ. Mean values ± 1 SEM for data obtained in four animals in each group are shown.

thesized sterol in the liver of the WHHL animals equaled only 310 nmol/g per hr which was significantly less than the value of 538 nmol/g per hr seen in the NZ control rabbits. Second, in contrast, there was a 5-fold increase in the content of [^{3}H]DPS in the adrenal glands of the WHHL rabbits (2215 nmol/g per hr) compared to the control animals (438 nmol/g per hr). The remaining organs showed similar levels of newly synthesized sterols in the two groups of rabbits although the values in the WHHL rabbits were somewhat lower in the colon, lung, and kidney. Notably, the content in quantitatively important tissues like the small intestine, skin, and muscle were essentially identical in these two experimental groups.

From these data, whole animal and whole tissue levels of newly synthesized sterol could be calculated and are shown in **Fig. 4.** As in the earlier study, the level of whole animal sterol synthesis was significantly higher in the NZ control rabbits (124.1 μmol/hr) than in the WHHL animals (85.3 μmol/hr). In both groups of animals, the great majority of the newly synthesized sterol was found in only four tissues: these included the liver, small bowel, skin, and carcass. Of these four tissues, the absolute content of [^{3}H]DPS was essentially identical in the small bowel, skin, and carcass of the control and WHHL animals. In contrast, the hepatic content of [^{3}H]DPS in the WHHL rabbits (21.3 μmol/hr per whole organ) was less than half of the content found in the NZ control rabbits (45.7 μmol/hr per whole organ). All of the other organs made small and essentially similar contributions to the total body synthesis rate seen in the two groups of rabbits. Hence, this study confirmed that the lower rate of whole animal sterol synthesis seen in the WHHL rabbits was due almost exclusively to a lower rate of sterol synthesis in the liver of these receptor-defective rabbits.

Sterol synthesis in vitro in the adult animals

While these in vivo measurements provided the most reliable rates of sterol synthesis, it is known that during

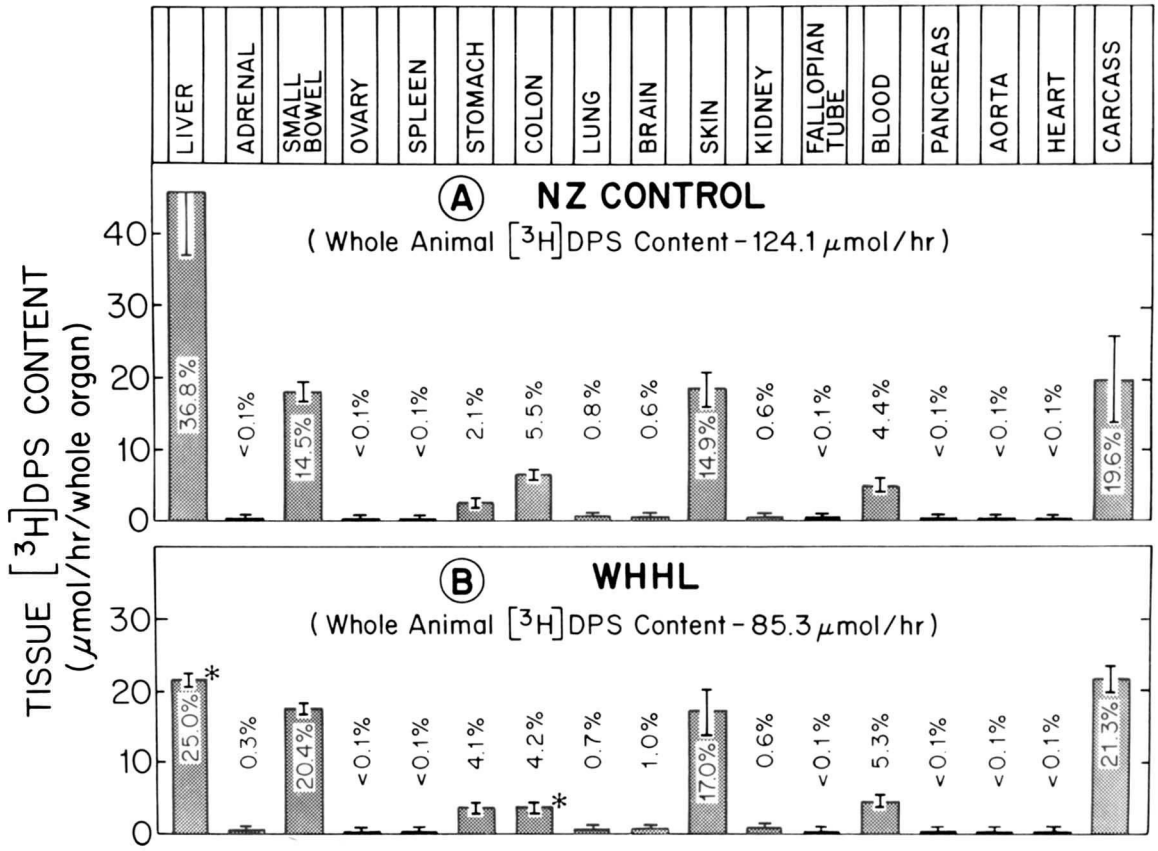

Fig. 4. The content of sterols newly synthesized in vivo per whole organ in 17 tissues of adult female rabbits. These data were derived from the experiment described in Fig. 3 by multiplying the content of [^{3}H]DPS found in each tissue times the weight of that same organ. These calculations gave the absolute content of [^{3}H]DPS in each organ, as shown on the vertical axis, and the sum of these values yielded the whole animal [^{3}H]DPS synthesis rates that are also shown for the NZ control and WHHL animals. In addition, the content of [^{3}H]DPS in each organ is also expressed as a percentage of the content of newly synthesized sterol found in the whole animals. In this figure, the carcass contained all of the muscle, fat, and skeleton of the animal. Mean values ± 1 SEM for data obtained in four animals are shown.

the 1-hr period of observation there are shifts of some newly synthesized sterol from one organ to another. In the rat (19) as well as in other species such as the monkey, hamster, guinea pig, and rabbit (15), the most notable movements of [^{3}H]DPS occur from the small bowel to the liver and from the liver to the blood. In order to be certain that there was not an unexpected and quantitatively important difference in the movement of [^{3}H]DPS among the different tissues of the WHHL animals, another experiment was undertaken in which rates of sterol synthesis were measured in vitro in the same organs taken from both NZ control and WHHL animals. As is apparent in **Fig. 5,** however, the results of these experiments were entirely comparable with those obtained in vivo. Sterol synthesis was significantly lower in liver slices (80 nmol/g per hr) and significantly higher in adrenal slices (113 nmol/g per hr) taken from the WHHL animals than in liver (113 nmol/g per hr) and adrenal slices (52 nmol/g per hr) obtained from the NZ control rabbits. Both the NZ control and WHHL animals manifested exceptionally high rates of sterol synthesis in the segment of small bowel proximal to the entrance of the common bile duct into the intestine (segment 1). The mean level of sterol synthesis in all segments of the small intestine in the two groups of animals averaged 263 ± 35 nmol/g per hr and 282 ± 43 nmol/g per hr, respectively. Sterol synthesis rates also were identical in the remaining organs of the body. Thus, these in vitro studies also showed a relatively lower rate of hepatic synthesis and a relatively higher rate of adrenal gland synthesis in the WHHL animals than in the controls.

While the results of these in vitro studies confirmed the relative differences in sterol synthesis rates seen in a given tissue in the NZ control and WHHL rabbits, it is apparent that there were variations between the in vitro and in vivo studies with respect to the absolute rates of synthesis as well as the relative rates expressed in the various organs of the same animal. Both of these effects would be expected on the basis of prior work. First, tissue slices or isolated cells studied in vitro generally give absolute rates of sterol synthesis that are well

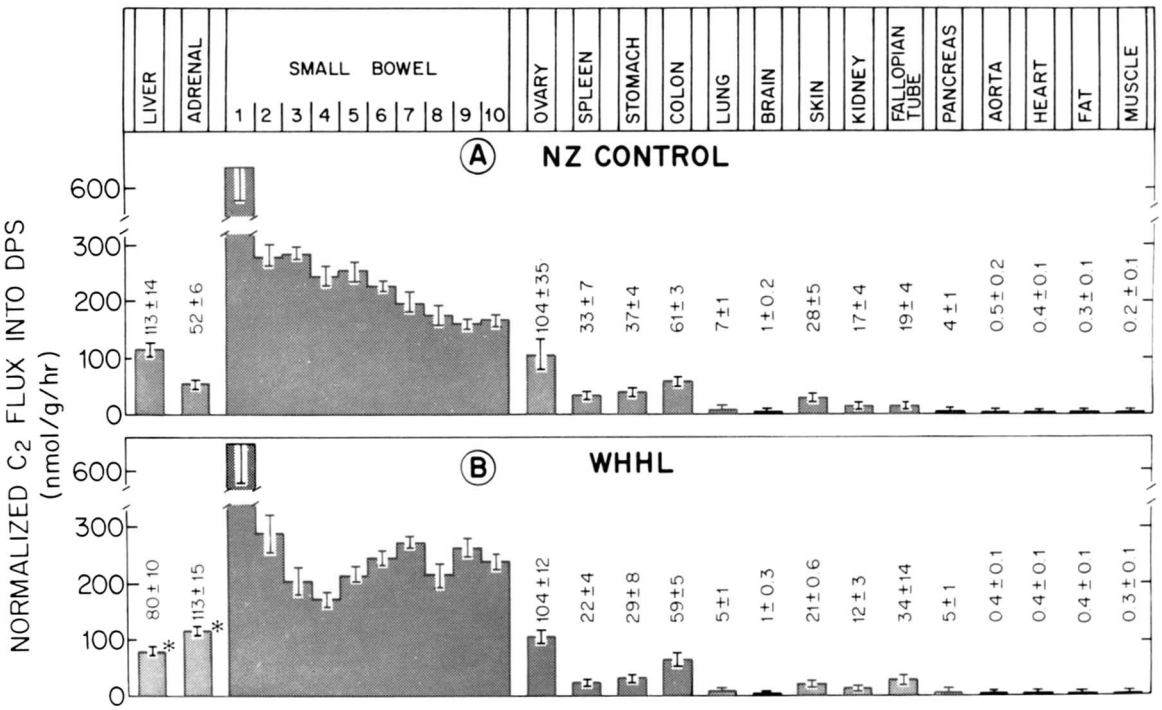

Fig. 5. Rates of sterol synthesis in vitro in 17 tissues of adult female rabbits. These animals were maintained on identical diets and under conditions of light cycling for 2 weeks prior to use. They were of similar age and were matched with respect to weight. The NZ control and WHHL animals weighed 2021 ± 50 g and 1960 ± 42 g, respectively. The animals were killed and tissue slices were prepared from each organ and from ten different areas of the small bowel, numbered from 1 to 10, proximal to distal. Aliquots of these tissue slices were incubated with [1-^{14}C]octanoate under in vitro conditions. The vertical axis shows the rate of acetyl CoA (C_2) incorporation into DPS per g of tissue per hr. Mean values ± 1 SEM are shown for three animals in each group. As in the preceeding figures, the asterisks indicate the rates in the WHHL rabbits that were significantly different from those in the NZ control animals. Rates of sterol synthesis in specific segments of the small bowel were also significantly different; however, since the mean rate of synthesis in the ten intestinal segments was not significantly different in the two groups of animals, no asterisk is shown for this tissue.

below rates obtained in the same tissue under in vivo conditions (18). Second, the specific activity of the [1-^{14}C]acetyl CoA pool generated from [1-^{14}C]octanoate is known to undergo essentially no dilution in the intestine, modest dilution in the liver, and very great dilution in most of the other tissues (23). Because of this the rank order of the tissues is skewed so that the intestine manifested the highest rate of C_2 flux into DPS while the liver and, particularly, the remaining tissues of the body exhibited relatively much lower rates.

Finally, samples of six tissues were taken from the same experimental animals for determination of contents of unesterified and esterified cholesterol. As shown in panels A and B of **Fig. 6,** the concentration of unesterified cholesterol was only marginally elevated in the liver, kidney, and lung. However, the cholesteryl ester concentration was elevated from 3- to 5-fold in these same tissues, while neither unesterified nor esterified cholesterol levels were elevated in the intestine or muscle. It is noteworthy that these three tissues that manifested significant elevations of cholesterol content were the same tissues in which reduced rates of cholesterol synthesis were manifest in vivo (Fig. 3).

It was possible that the higher levels of cholesteryl esters in these tissues was a result of an increased activity of the enzyme responsible for cholesterol esterification, acyl-CoA:cholesteryl acyltransferase (ACAT). ACAT activity, therefore, was measured in the microsomal fraction of the livers of these same animals. **Table 1** shows the cholesterol and cholesteryl ester contents of the livers and the microsomal fraction of the livers of the two groups of rabbits. The significant increase in cholesteryl ester content in the livers of the WHHL animals was emphasized by the 2.6-fold increase in the cholesteryl ester/cholesterol ratio; however, the relative amounts of free and esterified cholesterol in the microsomal fraction, the site of cholesteryl ester synthesis, were unchanged. There was also no significant difference in the ACAT activity in the control or WHHL groups.

DISCUSSION

The current studies demonstrate that tissues of the WHHL rabbit, which lacks nearly all LDL receptor activity, have normal or, in some cases, reduced rates of

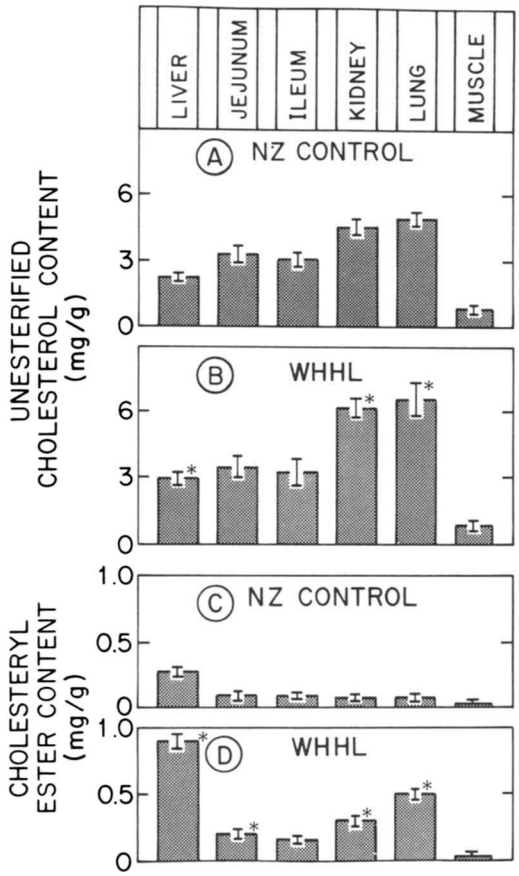

Fig. 6. Content of unesterified and esterified cholesterol in six tissues of the NZ control and WHHL animals. These tissue samples were obtained from the same animals described in the legend to Fig. 5. Duplicate aliquots of these organs were taken for independent determination of the content of unesterified and esterified cholesterol and are expressed as the mg of sterol per g wet weight of tissue. Mean values ± 1 SEM are shown for three animals in each group.

cholesterol synthesis. Only the adrenal gland of the 17 tissues examined manifested a significantly higher rate of cholesterol synthesis in the absence of LDL receptor activity. These findings were confirmed utilizing measurements of sterol synthesis in the different organs under both in vivo and in vitro conditions.

The current studies provide a number of observations that bear on the regulation of sterol synthesis in the normal and WHHL rabbit. The fetuses of the NZ control rabbits had a plasma cholesterol concentration that averaged only 79 mg/dl. After birth, however, their plasma cholesterol levels rose very significantly (Fig. 1) to reach values averaging over 400 mg/dl. This effect of a high intake of exogenous cholesterol from the milk of the mothers has previously been noted (24, 25) and presumably reflects the same limited capacity of the liver of the suckling animals to deal with a high dietary cholesterol intake as has been observed in the adult rabbit (26, 27). This increased intake of exogenous cholesterol was associated, nevertheless, with marked suppression of hepatic cholesterol synthesis (panel A, Fig. 2). Hence, in the suckling animals, the liver accounted for <10% of the sterol that was newly synthesized by the whole animal. Once weaned, the plasma cholesterol level returned to low values and sterol synthesis in the liver increased. Thus, as has been recently reported in other species such as the rat, monkey, guinea pig, and hamster, in the young adult animals in vivo the liver, small bowel, skin, and muscle of the carcass constituted the major sites for sterol synthesis in the whole animal (15).

The fetuses of the WHHL mothers already demonstrated significant hypercholesterolemia (Fig. 1), a finding which also has been reported in the fetuses of humans with FH (4). With the onset of milk intake, the plasma cholesterol level rose further and there was marked suppression of the rate of hepatic cholesterol synthesis just as was seen in the NZ control animals (panel A, Fig. 2). This finding is very likely a manifestation of the fact that while the WHHL animal cannot effectively transport LDL into tissues, its liver retains the ability to take up and metabolize remnants of chylomicrons (28). Hence, the suckling WHHL animal manifested essentially the same feedback regulation of hepatic sterol synthesis by dietary cholesterol as did the NZ control rabbit (14).

TABLE 1. Microsomal cholesterol content and ACAT activity in NZ control and WHHL rabbits

Experimental Group	Whole Liver			Liver Microsomes			
	Cholesterol Content (C)	Cholesteryl Ester Content (CE)	CE/C	Cholesterol Content (C)	Cholesteryl Ester Content (CE)	CE/C	ACAT Activity
	mg/g liver			*μg/mg protein*			*pmol/min per mg protein*
NZ control	2.2 ± 0.1	0.27 ± 0.1	0.12	39 ± 7	1.6 ± 0.2	0.041	7.6 ± 3.2
WHHL	2.9 ± 0.3	0.90 ± 0.3	0.31	60 ± 19	2.7 ± 0.7	0.045	6.1 ± 1.1

Microsomes were prepared from rabbits maintained on the low cholesterol diet, and microsomal esterified and unesterified cholesterol contents were estimated as described in Materials and Methods. ACAT assays were performed in duplicate or triplicate and the activity was calculated as pmol of cholesteryl oleate formed per min per mg microsomal protein. Means ± 1 SEM for three rabbits are shown in each group.

Once weaned and placed on a low dietary cholesterol intake, plasma cholesterol levels dropped markedly and the rate of hepatic cholesterol synthesis increased significantly in the NZ control animals. In the WHHL rabbit the plasma cholesterol continued to rise with weaning. However, since the amount of cholesterol entering the body carried in chylomicrons presumably did decline, there was also a significant increase in the rate of hepatic sterol synthesis in these animals with weaning. Nevertheless, in the face of the very high levels of plasma cholesterol carried in LDL, the livers of the WHHL rabbits did not reach the same level of cholesterol synthesis seen in the NZ control rabbits.

Hence, in the young adults, the levels of sterol synthesis in the two groups of rabbits illustrated in Figs. 3 and 4 were found to be essentially the same in most tissues. However, the synthesis of cholesterol in the liver and, to a lesser degree, in the colon, lung, and kidney, was suppressed below control values in the WHHL rabbits and was elevated only in the adrenal gland. The interpretation of these data is somewhat complex and depends upon the availability of information on two aspects of cellular LDL metabolism: these include *1*) identification of those differentiated tissues that actually take up LDL through receptor mediated means and *2*) the delineation of non-receptor-mediated mechanisms that may allow LDL uptake in specific tissues in the apparent absence of receptors. While definitive data are not available on either of these points, there is considerable information that indirectly bears on these two questions.

The first point deals with the identification of those tissues that actually rely on LDL uptake to supply a portion of their daily requirements for sterol. Under in vivo conditions, recent studies have shown that the liver of many species, including the rabbit, is quantitatively much less important to whole body sterol synthesis than previously believed (15, 19). The correlate of this finding is that much of the sterol required by nonhepatic tissues may be synthesized locally in those tissues. However, there is considerable variation among the different organs with respect to which tissues predominantly synthesize the cholesterol they require or, alternatively, which take it up from the blood. In the rat, for example, the great majority of sterol found in organs such as brain, skin, and muscle is synthesized locally whereas a much greater percentage of the newly synthesized sterol present in tissues like adrenal gland, spleen, kidney, and lung is apparently taken up from the blood after being synthesized in the liver (19).

A similar conclusion concerning the dependence of these different organs on uptake of plasma cholesterol can be derived from studies in which the drug 4-aminopyrazolo(3,4-d)pyrimidine was administered to rats to markedly reduce the levels of circulating lipoprotein cholesterol. This manipulation resulted in a significant increase in the rate of sterol synthesis, whether measured in vitro or in vivo (19, 29, 30), in tissues such as the adrenal gland, intestine, spleen, kidney, and lung suggesting that such organs normally took up and utilized lipoprotein cholesterol. In contrast, tissues such as skin, muscle, heart, and brain manifested little or no change in the rate of cholesterol synthesis suggesting that normally these latter organs took up little or no lipoprotein cholesterol and relied primarily on local synthesis to meet their daily requirements for tissue sterol turnover.

Taken together, these various observations suggest that the similar rates of sterol synthesis observed in muscle, fat, heart, aorta, carcass, and skin of the NZ control and WHHL rabbits in the present study was a consequence of the fact that these tissues normally synthesize essentially all of the sterol that they require for daily turnover and so take up little or no LDL cholesterol. Hence, the rate of sterol synthesis is essentially unaffected by deletion of the LDL receptor.

In the remaining tissues that presumably do utilize LDL cholesterol, the second question of importance concerns the quantitative relationships that exist between receptor- and non-receptor-mediated LDL uptake in each organ. In most animals in which the appropriate measurements have been made, non-receptor-mediated LDL clearance from the plasma accounts for about 30% to 50% of total LDL turnover (9, 10) and in the rabbit specifically equals 33% (11). This does not necessarily mean, however, that one-third of LDL cholesterol uptake in every organ is by non-receptor means. Conceivably, the relative importance of receptor- and non-receptor-mediated uptake could vary considerably among the different differentiated tissues. Studies using [^{14}C]sucrose-labeled LDL have shown that tissues such as liver, adrenal gland, spleen, intestine, and kidney are active in the uptake and degradation of LDL (31, 32). However, experiments have not yet been reported with [^{14}C]sucrose-labeled LDL that has been modified so that it no longer binds to the LDL receptor. Thus, no information is currently available as to the extent to which uptake in each organ is mediated by receptor and non-receptor mechanisms.

In a tissue where the great majority of LDL uptake is through receptor-mediated means, deletion of the receptor, as in the WHHL rabbit, would be expected to result in a significant increase in the rate of cholesterol synthesis. Possibly the adrenal gland represents such a tissue. On the other hand, if in another tissue non-receptor-mediated uptake is quantitatively more important to total LDL uptake, then deletion of the receptor would have little effect on sterol synthesis or

could actually be associated with suppression of the rate of cholesterol synthesis as the plasma LDL concentration reached very high levels. Whether such quantitative differences in the rates of receptor- and non-receptor-mediated LDL uptake can account for the observed enhanced rate of cholesterol synthesis in the adrenal gland and suppression of synthesis in the liver and other tissues in the WHHL rabbits must await quantitative measurements on the magnitude of each of these processes in all of the major organs of the rabbit.

These findings in the WHHL rabbit demonstrate that an extremely high concentration of plasma cholesterol can be maintained in the face of a low cholesterol diet and a low rate of whole animal cholesterol synthesis. Under these conditions the cholesterol content of various tissues is normal or only slightly elevated (Fig. 6). The isolated build-up of cholesterol in the LDL and IDL fractions of plasma results from the receptor defect, which is analagous to the accumulation of substrate behind a metabolic block. Once this plasma pool of cholesterol has built up, it can be maintained without high rates of cholesterol synthesis and/or absorption. Presumably this is due to the recycling of cholesterol in and out of cells via the non-receptor-mediated catabolism of LDL that must occur in the WHHL rabbit. Despite the high concentration of plasma cholesterol, tissue cholesterol content is normal in major tissues such as muscle and fat or is only slightly elevated as in the liver, lung, and kidney. Since the percentage of total body cholesterol that is present in the plasma is small, even in the WHHL rabbit, the rise in plasma cholesterol leads to only a slight increase in the total body content of cholesterol.

The authors wish to thank Joan Thorson, Debra Schentes, Edith Cole, Nancy Hammack, Leigh Riley, and Richard Gibson for their excellent technical assistance and Dorothy Dunham for her help in preparation of the manuscript. These studies were supported by research grants from the National Institutes of Health (HL 09610, AM 19329, and HL 20948) and by grants from the Moss Heart Fund.

Manuscript received 30 July 1982 and in revised form 17 December 1982.

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