Rates of sterol synthesis and uptake in the major organs of the rat in vivo

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Abstract This study was undertaken to determine the rates of sterol synthesis and uptake in the major organs of the female rat in vivo. At the mid-dark phase of the light cycle, control animals, animals in which hepatic sterol synthesis had been selectively inhibited by chylomicron infusion and animals in which the small intestine and liver had been surgically removed, were administered [^{3}H]water, and the content of ^{3}H-labeled digitonin-precipitable sterols ([^{3}H]-DPS) in different organs was measured 1 hr later. In control animals, the highest content of [^{3}H]DPS was found in the liver (2279 nmol/hr per g), adrenal gland (1222), ovary (791), and small bowel (529): the content of newly synthesized sterols was much lower in 13 other tissues. By selectively inhibiting sterol synthesis in the liver or by surgically removing the small intestine and liver, it was determined that of the total amount of [^{3}H]DPS synthesized in the whole animal about 50% had occurred in the liver, 24% in the small bowel, 8% in the skin, and 18% in the remaining tissues of the carcass combined. By analyzing the relationship between the content of [^{3}H]DPS in blood and in each organ, it was further possible to determine how much [^{3}H]DPS was synthesized and how much was taken up from the blood in each tissue. The highest rate of uptake was found in the adrenal gland where only 4% of the tissue content of [^{3}H]DPS came from local synthesis. Low rates of synthesis relative to the rates of uptake, were also found in the spleen (6%), lung (17%), and kidney (26%). In contrast, in other organs there was little uptake of [^{3}H]DPS from blood so that >75% of the [^{3}H]DPS present in brain and muscle, for example, was due to local synthesis. Lowering the circulating levels of plasma cholesterol markedly increased the synthesis of [^{3}H]DPS in tissues like adrenal gland, spleen, and kidney that were dependent upon plasma cholesterol as the major source of tissue sterols, but not in tissues such as muscle and brain. These studies have quantitated the importance of each major organ to total body synthesis and have delineated the rates of movement of [^{3}H]DPS between major tissue compartments of the rat. —**Turley, S. D., J. M. Andersen, and J. M. Dietschy.** Rates of sterol synthesis and uptake in the major organs of the rat in vivo. *J. Lipid Res.* 1981. **22:** 551–569.

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The general features of the regulation of rates of cholesterol synthesis in the whole animal and in man, and in individual tissues of the body have been known for a number of years (1). All, or nearly all, of the cholesterol synthesized within the body has been considered to come from either the liver or the small intestine, since only very low rates of sterol synthesis have been reported in the remaining tissues of the body in animals such as the rat, squirrel monkey, and, to the extent that data are available, man (2–4). Hepatic cholesterol synthesis is suppressed by cholesterol or bile acid feeding and fasting, and is markedly enhanced by diverting intestinal lymph away from the liver, by interrupting the enterohepatic circulation of bile acids, and by stress (5–9). The rate of sterol synthesis in the intestine is much less sensitive to the level of cholesterol in the diet, but is partially suppressed by prolonged fasting and is markedly enhanced by diverting the bile from the intestinal lumen (4, 10–12).

Many of the qualitative and quantitative conclusions concerning the importance of specific organs in whole body sterol synthesis have been based upon measurements of rates of incorporation of various ^{14}C-labeled substrates into cholesterol under in vitro conditions. Recent studies from this laboratory, however, have shown that there may be significant underestimation of true rates of sterol synthesis when such assay techniques are utilized. For example, the rates of incorporation of [^{14}C]acetate into digitonin-precipitable sterols (DPS) by liver and small bowel equals only about 45% of the true rates and in tissues such as skin, adrenal gland, ovary, lung, and muscle may correspond to only 4–25% of the true rates of sterol synthesis (13). Similar problems are encountered using [^{14}C]octanoate which gives rates of incorporation that are nearly equal to the true rates of synthesis in the liver (76%) and small bowel (91%), but which equals only about 6% of the true rate of sterol synthesis in muscle (13). From results such as these it is

Abbreviations: DPS, digitonin-precipitable sterols; APP, 4-aminopyrazolo(3,4-d)pyrimidine; S.A., specific activity; RBV, residual blood volume; RBC, red blood cells; LDL, low density lipoprotein; HDL, high density lipoprotein.

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apparent that rates of sterol synthesis have been disproportionately underestimated in nonhepatic, nonintestinal tissues utilizing such in vitro assay techniques and it follows, therefore, that such tissues may be quantitatively more important in terms of total body synthesis than has heretofore been appreciated.

In an attempt to circumvent these problems, recent work has been published utilizing the incorporation of [^{3}H]water into DPS as a measure of rates of sterol synthesis in vivo (13–16). [^{3}H]Water rapidly penetrates most cell membranes so that a constant specific activity is achieved in total body water very rapidly. Furthermore, as long as the animal is not given any fluids, this specific activity remains constant for many hours. The ^{3}H of [^{3}H]water is incorporated into the sterol molecule either directly or after equilibration with the reductive H of NADPH (17). Under in vivo conditions, this incorporation of [^{3}H]water into DPS occurs as a linear function of time and so can be used to quantitate rates of sterol synthesis. Furthermore, since it has been determined that approximately 22 μg atoms of ^{3}H are inserted into each μmol of cholesterol, at least in the liver and small intestine, rates of [^{3}H]water incorporation into sterols can be converted to absolute rates of cholesterol synthesis (13).

Utilizing this technique, recent investigations have shown that in control rats only about half of the sterol synthesized over a 1-hr period is found in the liver; the remainder is found in the various tissues of the carcass (16). When such animals are fed cholesterol or fasted, hepatic sterol synthesis is suppressed so that approximately 80% of sterol synthesis taking place in the whole animal occurs in the extrahepatic tissues of the carcass (16). Thus, these recent studies not only provide new information on the regulation of sterol synthesis in the liver and carcass under in vivo conditions, but, more importantly, support the concept that extrahepatic tissues are relatively more important sites for sterol synthesis than has heretofore been appreciated.

The current studies were undertaken to provide more detailed information on the rates of sterol synthesis in the individual tissues of the carcass and on the rates of transfer of newly synthesized cholesterol from the liver to these organs. Using a combination of studies in which there was selective deletion of the hepatic and intestinal contribution to total body sterol synthesis, new information was obtained concerning *1*) the content of newly synthesized cholesterol in all of the major tissues of the rat, *2*) the contribution of each of these tissues to whole animal sterol synthesis, *3*) the rate of transfer of newly synthesized cholesterol from the liver to each of the major organs of the carcass, and *4*) the mechanisms of regulation of sterol synthesis in the extrahepatic tissues under in vivo conditions.

MATERIALS AND METHODS

Animal preparations

The female Sprague-Dawley-derived rats used in these studies were purchased in the weight range of 150–180 g (Charles River Breeding Laboratories, Wilmington, MA) and were housed in colony cages in an isolated room and subjected to light-cycling for approximately 2 weeks before use (7). During this time they were allowed free access to water and Formulab rat chow diet (Ralston Purina Company, St. Louis, MO). Following this period of light-cycling, the animals were subjected to several different experimental manipulations before they were used to quantitate rates of sterol synthesis in various organs.

Experimental manipulations of animals

Several studies were carried out on fed control animals. Such animals had completed their period of light-cycling and had been allowed free access to water and chow diet. The rats were administered [^{3}H]water and were killed at the mid-dark period of their light cycle when their stomachs were filled with food.

In a second type of experiment, the rate of cholesterol synthesis in the liver was suppressed to varying degrees by the acute intravenous administration of cholesterol carried in chylomicrons. For these studies chylomicrons were harvested from donor rats fitted with intestinal lymphatic cannulae. The chylomicrons were isolated and washed by centrifugation as previously described (6, 18, 19). Fed control rats that had been light-cycled for 2 weeks were then administered, intravenously, varying amounts of this lipoprotein fraction containing 0 to 50 mg of cholesterol. The chylomicrons were infused as a bolus over a 20–30-min period at the mid-light phase of the light cycle and the animals were then returned to colony cages in the light-cycling room. Twelve hr later, at the mid-dark phase of the light cycle, the animals were administered [^{3}H]water for determination of rates of cholesterol synthesis.

In a third type of experiment, rats were subjected to acute surgical removal of the small bowel or the liver and small bowel. Using animals at the mid-dark period of the light cycle, each rat was anesthetized with diethyl ether and a catheter was placed in a tail vein. The abdomen was opened and the entire small bowel was excised by cutting along the margin of the intestine from the ileocecal valve to the ligament of Treitz. Care was taken to preserve as much of the

portal circulation as possible. When tested in vitro, hepatic sterol synthesis was the same in control animals and in rats subjected to enterectomy 1 hr before. In some animals the liver was also removed. A ligature was placed around the base of each of the major divisions of the liver and the lobes were removed sequentially. As much liver was removed as possible without interfering with blood flow through the inferior vena cava. However, approximately 0.4 to 0.5 g of liver tissue (of a total liver weight of approximately 7.5–8.0 g) usually had to be left in the animal. The abdomen was then closed in two layers. The animal was placed in an individual restraining cage and a thermocouple was inserted into its rectum. Operated control animals were subjected to the same procedures except that the small bowel and liver were left intact after manipulation. Immediately after being placed in the restraining cage, each rat was given 50 mg of glucose intravenously and an infusion was begun through the tail vein catheter at a rate of 1.2 ml/hr. The infusate contained 9 g of NaCl and 2 g of glucose per liter. Both the control and eviscerated animals were then placed in plastic chambers under fume hoods: the air temperature in the chambers was maintained electrically at 34–35°C. Approximately 35–45 min later, when they were fully awake, actively moving in the restraining cages, and had body temperatures of 37.5–38°C, the animals were injected intravenously with [^{3}H]water.

In a fourth type of experiment, rats were treated with 4-aminopyrazolo(3,4-d)pyrimidine (APP) to suppress lipoprotein secretion from the liver and lower plasma cholesterol levels (20). Since this drug causes the animals to stop eating, both the control group of rats and the drug-treated animals were subjected to fasting using a protocol previously described in detail (21). One group of animals received APP, 20 mg/kg body weight, intraperitoneally in 25 mM phosphate buffered-isotonic saline (pH 4). Control animals received equivalent volumes of buffer. These injections were administered daily on four successive mornings. During this time the animals were allowed access to drinking water but received no food. Approximately 12–15 hr before the termination of the experiment, each animal was fitted with a tail vein catheter and placed in an individual restraining cage. During the subsequent period of 12–14 hr, all animals were infused intravenously at a rate of 1.2 ml/hr with a solution containing NaCl (77 mM), K_2HPO_4 (8.3 mM), KCl (16.7 mM), and glucose (370 mM). At the end of this period, at the mid-dark period of the light cycle, the intravenous infusions were stopped and each animal was administered [^{3}H]water for determination of rates of sterol synthesis.

Determination of sterol synthesis rates in vivo

After these various experimental manipulations were carried out, the rats were placed in restraining cages, fitted with tail vein catheters (if not already in place), and administered [^{3}H]water intravenously (usually 100 mCi) in 0.5–1.0 ml of isotonic NaCl solution. The catheters were then immediately removed and the animals were kept in the restraining cages under fume hoods until killed 1 hr later: they were not allowed any food or water during this period. Sixty min after the injection of the [^{3}H]water, each rat was anesthetized with diethyl ether. The abdomen was immediately opened and 5.0 ml of blood was aspirated from the abdominal aorta into a heparinized syringe. Various organs were then removed, weighed, and cut into slices 2–3 mm thick with a razor blade as quickly as possible. The slices were rinsed briefly in cold 0.9% sodium chloride solution to remove excess blood and were blotted on filter paper. Two or three aliquots of these slices from each organ were then weighed on an electric balance and placed in glass tubes for saponification. For the larger organs these tissue aliquots weighed 400–800 mg: for the smaller organs, e.g., adrenal gland and ovary, the whole tissue was used to determine radiolabeled sterol content. The following tissues were sampled in most experiments: liver, stomach, proximal small bowel (proximal half of small bowel), distal small bowel (distal half of small bowel), colon (mid-transverse colon), heart, lungs, pancreas, adrenal gland, ovary, kidney, spleen, adipose tissue (retroperitoneal fat), muscle (medial thigh muscle), brain (cerebrum), and whole blood. After removal of these organs the animal was skinned: this entire tissue (hair, skin, and subcutaneous fat) was weighed and saponified. The remaining carcass of the animal containing principally muscle, bone, and marrow was also weighed and, along with the washed-out contents of the gastrointestinal tract, was saponified. In a few studies the animal tissues were separated only into liver, small bowel, blood, and carcass; in these cases the "carcass" contained all tissues other than the liver, small intestine, and 5.0 ml of blood. A portion of the whole blood sample was centrifuged to yield plasma from which the specific activity (S.A.) of plasma water was determined. It should be noted that since no tissue was discarded, "whole body" sterol synthesis rates could be calculated as the sum of the synthetic rates found in each tissue compartment.

Determination of sterol synthesis rates in vitro

In several experiments, rates of sterol synthesis in the liver and small bowel also were measured in vitro. At the time the animals were killed, aliquots of these

two organs were taken for determination of the rate of [^{3}H]water incorporation in vivo into sterols as described above. Other aliquots were taken and cut on a mechanical tissue slicer into slices 0.8 mm thick (7, 22, 23). These slices were rinsed in cold 0.9% NaCl solution and blotted on dampened filter paper. Six aliquots of these slices, each weighing 300 mg, were placed in center-well flasks containing 5.0 ml of Krebs' bicarbonate buffer and Na octanoate. The concentration of the octanoate was 1.0 and 1.5 mM, respectively, in the flasks containing liver and intestine. Three of these six flasks also contained 10 μCi of [1-^{14}C]octanoate. The flasks were then gassed with 95% O_2: 5% CO_2 and incubated in metabolic shakers at 37°C for 1.5 hr. The liver slices were shaken at 160 oscillations/min while the intestinal slices were incubated at 120 oscillations/min. After processing, the three flasks containing no [1-^{14}C]octanoate were used to correct for any [^{3}H]DPS present in the tissue that would be precipitated and quantitated with the [^{14}C]DPS. In practice, however, this correction proved to be minimal since the radioactivity present in this group of flasks (when counted in the ^{14}C channel) was less than 1% of that present in the slices incubated with [1-^{14}C]octanoate. In the case of the liver, four additional flasks containing 1.0 mM [1-^{14}C]octanoate were also run in parallel in order to determine the specific activity of the ketone bodies synthesized under these in vitro conditions (22).

Determination of tissue residual blood volumes

Even though tissue samples were sliced and washed prior to assaying their content of [^{3}H]DPS, there was still a small amount of blood contaminating each organ. In preliminary experiments we found that the [^{3}H]DPS present in whole blood 1 hr after administration of [^{3}H]water was almost equally distributed between the plasma lipoproteins and the red blood cell membranes. We chose, therefore, to measure the volume of whole blood contained in each organ and to use this volume to correct the tissue content of [^{3}H]DPS for the amount of [^{3}H]DPS present in the retained blood. Corrections based upon measurement of plasma volumes (using ^{125}I-labeled bovine serum albumin) gave essentially identical results. In order to make these measurements, 2-ml aliquots of whole rat blood were incubated with 10 μCi of ^{51}Cr ($Na_2{}^{51}CrO_4$) for 20 min at room temperature (24). The red blood cells (RBC) were then washed three times with 0.9% NaCl solution containing 0.5 g/dl of bovine serum albumin and brought to a final volume of 2.0 ml. Each rat was administered 0.5 ml of this [^{51}Cr]RBC suspension intravenously and was killed 10 min later. The tissues were removed, sliced, washed, and weighed as described above. The aliquots of each tissue and of whole blood were solubilized and assayed for ^{51}Cr content (24).

Analytic procedures

We have previously shown that special precautions are required for isolating digitonin-precipitable sterols in the presence of large amounts of [^{3}H]water and this method is described in detail elsewhere (16). The major steps may be outlined as follows. The aliquots of tissue obtained from the in vivo studies (DPS labeled with ^{3}H) or the in vitro incubations (DPS labeled with ^{14}C) were saponified with alcoholic KOH. The sterols were then extracted quantitatively and were dried under vacuum, and the digitonides were split with pyridine (25). The free sterols were extracted with diethyl ether, dried under vacuum, and assayed for ^{3}H or ^{14}C content.

In several experiments the specific activity of free and esterified cholesterol in various organs was determined. The slices of tissue were first extracted on a steam bath with chloroform–methanol 2:1 (v/v) and the free and esterified sterols were separated on silicic acid–celite columns (21). After saponification, the sterols were precipitated as the digitonides and aliquots were taken for determination of ^{3}H content and mass.

Calculations

Several types of calculations were carried out in these studies. In the in vivo experiments it was necessary to determine the mean specific activity of body water throughout the 1-hr interval over which rates of sterol synthesis were being measured. As previously described (16), this value, expressed as cpm ^{3}H/nmol 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 specific activity of plasma water determined at 1 hr after injection of the [^{3}H]-water to the mean specific activity of body water present throughout the 1-hr period of time. The term 0.92 corrects the calculation for the concentration of water determined in these studies to be present in 1.0 ml of plasma.

Rates of sterol synthesis (or 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/g) and were calculated using the following relationship:

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

In most instances 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). This value was calculated by dividing the product of the weight of the organ and the rate of sterol synthesis (or content) by 1000.

In some in vivo studies, the residual blood volumes present in each tissue, expressed as the mg of blood present in 1 g of tissue (mg/g), were calculated by dividing the cpm of ^{51}Cr present in 1 g of a particular organ by the concentration of ^{51}Cr present in 1 mg of whole blood (cpm/mg). Using the concentration of [^{3}H]DPS per g of whole blood and this value for the residual blood volume, one could then calculate that portion of the tissue [^{3}H]DPS content that was present in the blood still contaminating the tissue. Corrected values for the tissue content of [^{3}H]DPS were then calculated using the following relationship:

$$\frac{\text{(nmol/hr/g of [}^3\text{H]DPS content)} - \text{(nmol/hr/g of [}^3\text{H]DPS in RBV)}}{1 - (\text{RBV}/1000)}$$

In one group of in vivo studies, rates of [^{3}H]-DPS synthesis (or content) were not determined in every organ as described in detail above. Rather, the content of [^{3}H]DPS was measured in only four tissue compartments, i.e., liver, small intestine, whole blood, and remaining carcass. In these studies, the weight of the whole animal, liver, and small intestine was determined directly when each animal was killed. The weight of the whole blood volume was calculated from the weight of each animal using the mean density of rat blood and assuming that there was 6.40 ml of blood per 100 g body weight in the rat (26). The weight of the carcass was calculated by subtracting the weights of the liver, small intestine, and 5.0-ml blood sample from the whole animal weight. The volume of blood remaining in the carcass at the time the assays were carried out was calculated by subtracting the 5.0-ml volume of blood aspirated from the aorta from the calculated total volume of blood in the animal. The [^{3}H]DPS content of the liver, small intestine, and whole blood was determined directly. However, the radiolabeled sterols present in the carcass had to be corrected for the [^{3}H]DPS present in the residual blood volume that was necessarily processed with the other tissues of the carcass. This was accomplished by subtracting the product of the calculated volume of blood remaining in the carcass and the concentration of [^{3}H]DPS per ml of whole blood from the total radioactivity in the carcass.

In the studies involving in vitro assays of rates of sterol synthesis, synthetic rates were calculated in terms of the nmol of C_2 units (acetyl CoA units) incorporated into DPS per hr per g of tissue (nmol/hr per g). These rates were corrected for dilution of the specific activity of the intracellular [1-^{14}C]acetyl CoA pool derived from the oxidation of [1-^{14}C]octanoate. These values were calculated by means of the following relationship (22):

$$\frac{\text{(cpm [}^{14}\text{C]DPS) (6)}}{\text{(1.5 hr) (g tissue weight) (S.A. [}^{14}\text{C]octanoate) (F)}}$$

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 the conversion of [1-^{14}C]-acetyl CoA to sterols (22). The factor F corrects for dilution of the specific activity of the intracellular [1-^{14}C]acetyl CoA pool derived from [1-^{14}C]octanoate. In the case of liver slices, this factor equals approximately 0.72 and was determined experimentally as the ratio of the actual specific activity of the acetyl CoA pool (determined from the specific activity of the newly synthesized ketones) to the theoretical specific activity that the pool should have if undiluted by unlabeled acetyl CoA units (determined from the specific activity of the [1-^{14}C]octanoate). In the case of intestinal slices this factor equals 0.91 and was determined as the ratio of the incorporation rate of acetyl CoA units into DPS from [1-^{14}C]octanoate to the rate of acetyl CoA units incorporated into DPS determined with [^{3}H]water (13).

Where appropriate, mean values ± 1 SEM for groups of data are given. For correlating two variables, linear regression lines were fitted by the method of least squares to the data obtained from individual animals and have the usual form of $y = a + bx$.

RESULTS

In previously published experiments (16) and in other preliminary studies, we have found that the rate of [^{3}H]water incorporation into DPS in vivo is a linear function of time for over 1 hr in the liver, the small intestine, and the other major tissues of the carcass. Initial experiments, therefore, were undertaken to determine how much [^{3}H]DPS was present in the tissues of fed rats administered [^{3}H]water at the mid-dark point of the light cycle and killed 60 min later. Results of these studies are illustrated in **Fig. 1.** The upper panel shows the amount of [^{3}H]DPS present in 1 g of each tissue (expressed as the nmol of [^{3}H]-water incorporated into DPS per hr per g). The highest content of [^{3}H]DPS was found in the liver (2279 nmol/hr per g), adrenal gland (1222 nmol/hr

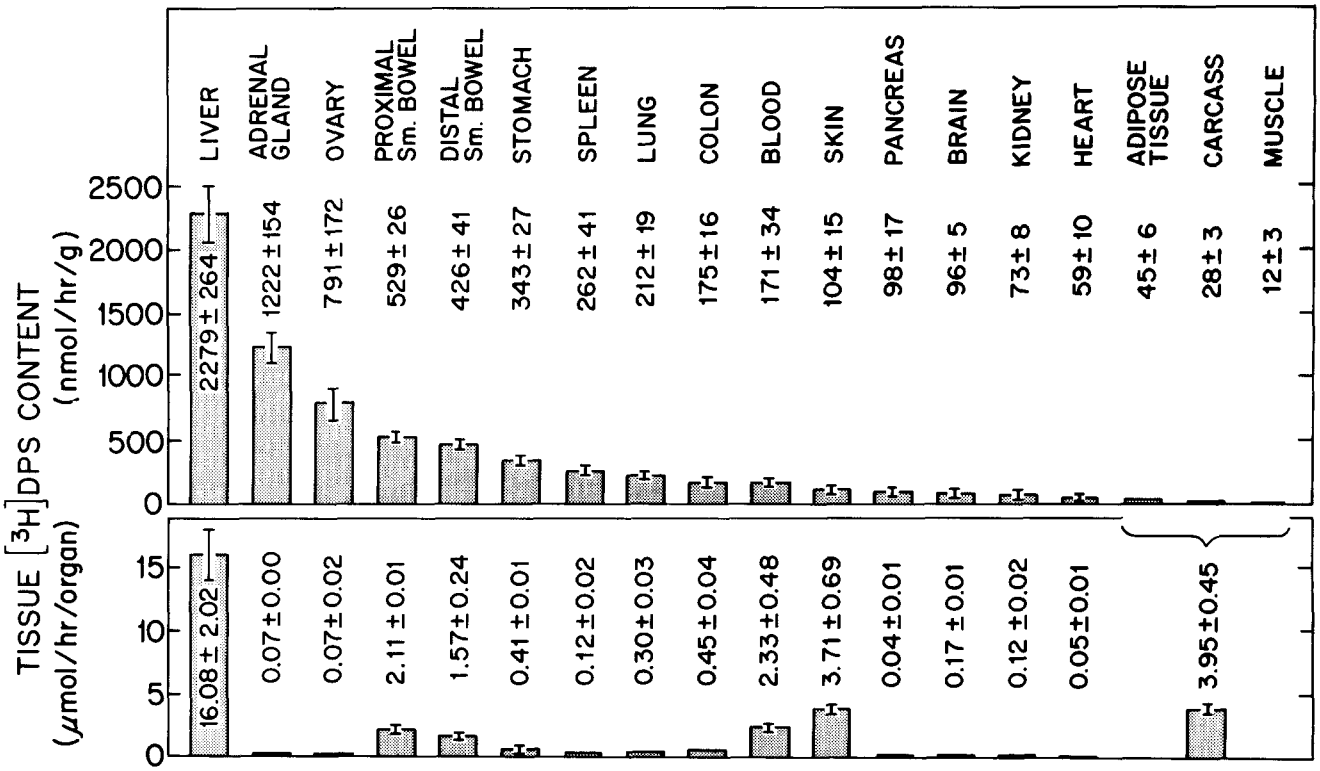


Fig. 1. Distribution of newly synthesized sterols in various tissues of the rat. These animals were killed 1 hr after they were administered [^{3}H]water and the tissues were removed, weighed, and assayed for the amount of ^{3}H in the digitonin precipitable sterols (DPS). These rates are expressed as either the nmol (upper panel) or μmol (lower panel) of [^{3}H]water incorporated into the DPS fraction per hr per g of tissue (upper panel) or per whole organ (lower panel). In the upper panel the [^{3}H]DPS content per g of tissue is shown for adipose tissue, muscle, and the residual carcass: in the lower panel, these three tissues are combined. The animals in this study had a mean weight of 211 ± 5 g. Total body synthesis of DPS (the sum of all values in the lower panel) equalled 31.55 μmol/hr. The data represent the mean ± 1 SEM values obtained in four animals.

per g), and ovary (791 nmol/hr per g). The small intestine and stomach had the next highest contents while the remaining organs of the abdomen and thorax and the skin and brain contained significantly lower amounts of [^{3}H]DPS. The lowest content of [^{3}H]DPS was found in the heart (59 nmol/hr per g), adipose tissue (45 nmol/hr per g), and striated muscle (12 nmol/hr per g). In this panel the tissue designated as "carcass" contained principally striated muscle, bone, and marrow. It should also be noted that the blood DPS contained 171 nmol of [^{3}H]water at the end of 1 hr.

The weight of the organs in these animals is given in **Table 1.** When these weights were multiplied by the amount of [^{3}H]DPS present in 1 g of each tissue, the content of [^{3}H]DPS in each whole organ 1 hr after administration of the [^{3}H]water was obtained (expressed as the nmol of [^{3}H]water incorporated into DPS per hr per organ), and these values are shown in the lower panel of Fig. 1. The liver had the highest amount and contained DPS in which 16.08 μmol of [^{3}H]water had been incorporated in 1 hr. The next highest contents were found in the carcass (3.95 μmol/hr), skin (3.71 μmol/hr), small intestine (3.68 μmol/hr), and blood (2.33 μmol/hr). The other organs all contained much lower amounts of [^{3}H]DPS. The sum of all of these values gives a whole-animal sterol synthesis rate equivalent to the incorporation of 31.55 μmol of [^{3}H]water into DPS per hr. Thus, of this total content of [^{3}H]DPS, 51% was found in liver, 13% in carcass, 12% in skin, 12% in small intestine, and 7% in blood; the remaining 5% of [^{3}H]DPS was distributed among the other organs.

In an identically treated group of animals, the absolute specific activity of unesterified cholesterol in the major organs was measured. The highest value was found in the liver and equalled approximately 20,000 cpm/mg of sterol. The specific activity of unesterified cholesterol was measured in eight other organs in each animal and these values are shown in **Fig. 2** relative to the value found in hepatic unesterified cholesterol.) One hr after administration of [^{3}H]water, the specific activity of plasma unesterified cholesterol was about 74% of that in the liver, while the specific activity of free cholesterol in the small bowel, adrenal gland, ovary, and whole blood was

TABLE 1. Mean organ weights in the rats used in these experiments

Tissue	Weight
	g
A. Whole animal	211 ± 5.10
B. Liver	7.05 ± 0.40
C. Adrenal gland	0.06 ± 0.00
D. Ovary	0.09 ± 0.01
E. Proximal small intestine	4.02 ± 0.25
F. Distal small intestine	3.32 ± 0.25
G. Stomach	1.13 ± 0.05
H. Spleen	0.44 ± 0.03
I. Lung	1.40 ± 0.10
J. Colon	2.58 ± 0.17
K. Blood	13.50 ± 0.33
L. Skin	37.20 ± 1.40
M. Pancreas	0.46 ± 0.09
N. Brain	1.71 ± 0.02
O. Kidney	1.62 ± 0.18
P. Heart	0.75 ± 0.02
Q. Carcass	136 ± 6.12

This table lists the mean animal and organ weights for the experimental animals used in the studies shown in Fig. 1. For the purposes of this table the small intestine was divided into proximal and distal halves. The blood volume for the rat was assumed to equal 6.40 g per 100 g body weight (26). The carcass (Q) contained all tissues, principally muscle, bone, and marrow, not otherwise listed in this table along with the contents of the gastrointestinal tract. The values represent the means ± 1 SEM for four rats.

much lower, averaging only 25% to 33% of that found in the liver. The specific activity of free cholesterol in muscle, kidney, and brain was very much lower and equalled <3% of the specific activity of unesterified cholesterol in the liver. The specific activity of the esterified cholesterol fraction in most of these tissues was so low that it could not be accurately measured in these particular studies.

Thus, these initial experiments provided data on the content of newly synthesized DPS in the various tissues of the rat and on the specific activities of these newly synthesized sterols. The critical issue, however, was to delineate how much of the [^{3}H]DPS present in each tissue was synthesized de novo in that organ and how much was taken up from the blood either by uptake of cholesterol carried in specific lipoproteins or by unidirectional molecular diffusion. This problem was investigated using two entirely different animal models. In one, the contribution of the liver to total body sterol synthesis was eliminated by suppressing the rate of hepatic cholesterol synthesis through the intravenous administration of cholesterol carried in chylomicrons. In the second model, the effect of the acute surgical removal of the small bowel and of the small bowel and liver on the content of [^{3}H]DPS in the other tissues of the rat was investigated.

The first model took advantage of the well-known observation that the remnants of chylomicrons are

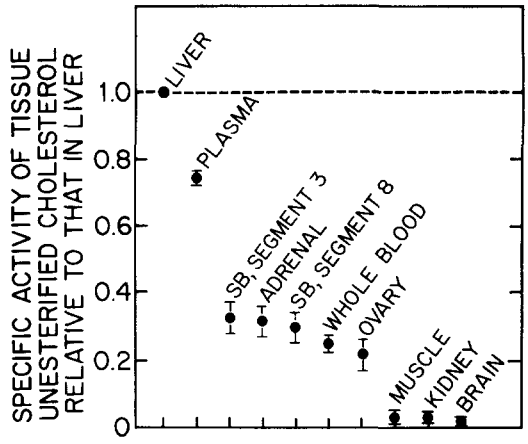


Fig. 2. Relative specific activities of unesterified cholesterol in various tissues of the rat. These tissue specimens were obtained from animals using the protocol described in the legend to Fig. 1. The sterols were extracted from the tissues, the free and esterified cholesterol were separated by column chromatography, and the specific activities were determined. In this diagram the absolute specific activity of the unesterified cholesterol in each tissue was divided by the absolute specific activity of the unesterified cholesterol found in the liver of the same experimental animal. In actuality, the absolute specific activity of hepatic free cholesterol averaged about 20,000 cpm per mg of cholesterol. Values are given for both mid-jejunum (SB segment 3) and mid-ileum (SB segment 8).

cleared nearly entirely in the liver (18, 27, 28). Thus, after the intravenous administration of this lipoprotein particle there is a marked rise in the content of cholesteryl esters and a reciprocal suppression of the rate of cholesterol synthesis in the liver but not in other tissues of the body (18). This effect was reproduced in this first group of investigations, as illustrated in **Fig. 3**. When chylomicrons containing from 9 to 50 mg of cholesterol (from 720 to

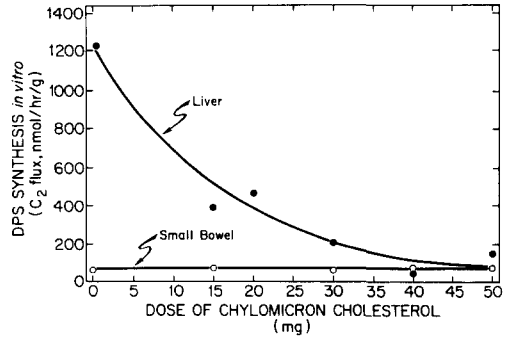


Fig. 3. Rates of hepatic and intestinal sterol synthesis, measured in vitro, in animals administered cholesterol carried in chylomicrons. In this study the animals were administered varying amounts of chylomicrons intravenously and then killed 12 hr later at the mid-dark point of the light cycle. Aliquots of liver and small intestine were removed and used to assay rates of sterol synthesis in vitro. In this diagram the rate of incorporation of acetyl CoA units into DPS is plotted against the amount of chylomicron cholesterol administered to each animal. Each point represents the mean of data obtained in 2–7 animals.

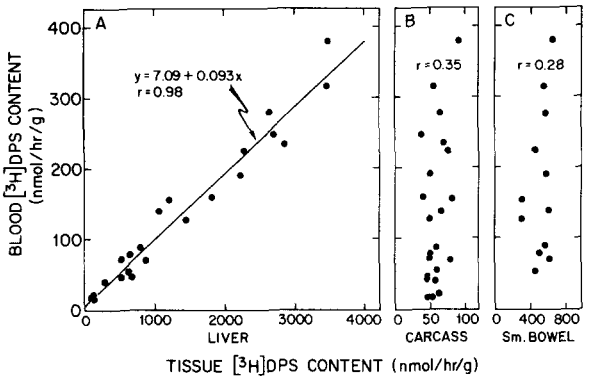


Fig. 4. Relationship between the amount of newly synthesized sterols appearing in blood and the content of [^{3}H]DPS in the liver, carcass, and small bowel under in vivo conditions. The rats used in this study were administered intravenously different amounts of cholesterol carried in chylomicrons in order to suppress to varying degrees the rate of hepatic cholesterol synthesis. Twelve hours later, they were administered [^{3}H]water and the content of [^{3}H]DPS was assayed 1 hr later in the liver, blood, small intestine, and remaining tissues of the carcass. In this diagram the content of [^{3}H]DPS in the blood of each of the animals is plotted against the content of [^{3}H]DPS in the liver (Panel A), carcass (Panel B) and small bowel (Panel C) of the same rat.

4000 mg of triglyceride) were administered to animals at the mid-light point of the light cycle and rates of sterol synthesis were assessed 12 hr later using an in vitro technique, there was a marked drop in the rate of DPS synthesis in the liver but no change in the rate of sterol synthesis in the small bowel. In such animals, the total plasma cholesterol levels were in the normal range of 62–75 mg/dl except in the animals receiving the highest dose of cholesterol (50 mg), where all of the chylomicrons had not yet been cleared. Utilizing such animals, the content of [^{3}H]DPS in the major tissue compartments 1 hr after the administration of [^{3}H]water was quantitated under circumstances where the rate of hepatic sterol synthesis had been selectively inhibited to varying degrees by the administration of chylomicrons 12 hr before. For the purposes of this study, the content of [^{3}H]DPS was measured in only four tissue compartments, i.e., liver, small bowel, whole blood, and carcass. In this instance, the carcass contained all of the tissues of the body other than the liver, small bowel, and blood.

The data obtained from these experiments were used to assess three specific questions. The first question dealt with the tissue source(s) of the [^{3}H]DPS that appeared in the blood 1 hr after the administration of [^{3}H]water. In order to address this question, the content of [^{3}H]DPS in the blood of each animal was plotted against the content of [^{3}H]DPS found in the liver, carcass, and small bowel of the same animal, as shown in **Fig. 4.** In Panel A it is apparent that there is a direct correlation between the level of [^{3}H]DPS in the blood and the content of [^{3}H]DPS in the liver (r = 0.98). Furthermore, the intercept on the y axis was not significantly different from 0 suggesting that when hepatic [^{3}H]DPS content decreased to 0, little or no [^{3}H]DPS entered the blood from the other two tissue compartments, i.e., small intestine and carcass. This conclusion was supported by the observations in Panels B and C showing that there was essentially no correlation between the content of [^{3}H]DPS in the carcass (r = 0.35) or small bowel (r = 0.28) and the amount of [^{3}H]DPS appearing in blood. From the mathematical relationships between the content of [^{3}H]DPS in the blood and liver shown in panel A, it can be calculated that in a control animal (not injected with chylomicrons) the liver contributed an amount of newly synthesized DPS to the blood compartment containing at least 2.74 μmol of [^{3}H]water (2300 nmol/hr per g times 0.093 times 6.4 g/100 g body weight times 2 divided by 1000 nmol/μmol).[2] In contrast, assuming that the intercept on the y axis was significantly different from 0 (which it was not), the maximum amount of [^{3}H]DPS that could have been contributed to the blood compartment from the carcass and small bowel would have equalled only about 0.09 μmol/hr (7.09 nmol/hr per g times 6.4 g/100 g body weight times 2 divided by 1000 nmol/μmol).[2]

Since all, or nearly all, of the [^{3}H]DPS present in blood was derived from hepatic sources, it was likely that some of the [^{3}H]DPS present in the carcass and small intestine had been taken up from the blood and so had ultimately been derived from the liver. Thus, the second question addressed by this experiment concerned the amount of [^{3}H]DPS synthesized in the liver that moved into the blood and was taken up by the small intestine and other tissues of the carcass.

To determine the quantitative significance of this transfer process, the content of [^{3}H]DPS in the carcass and small bowel of each animal was plotted against the content of [^{3}H]DPS in the liver of the same animal. As seen in Panels A and B, respectively, of **Fig. 5**, the [^{3}H]DPS content of the carcass and small bowel was nearly independent of the content of [^{3}H]DPS in the liver and, consequently, in the blood. Thus, while there was uptake of a small amount of [^{3}H]DPS into these two tissues from the blood, most of the [^{3}H]DPS must have been derived from de novo sterol synthesis in the carcass and small bowel. From the mathematical relationships shown in Fig. 5, both the minimum syn-

[2] For the calculations carried out in this set of experiments, the following values were utilized: liver [^{3}H]DPS content, 2300 nmol/hr per g; blood volume, 6.4 g/100 g body weight; animal body weight, 200 g; liver weight, 7.2 g; carcass weight, 170 g; small bowel weight, 7.1 g.

thesis rates and minimum uptake rates in these two tissue compartments can be calculated. Under circumstances where the hepatic (and blood) content of [^{3}H]DPS was 0, the carcass and small bowel content of [^{3}H]DPS equalled 54.4 and 468 nmol/hr per g, respectively. From these values the minimal rates of sterol synthesis in the carcass and small bowel must have been equal to the incorporation of 9.3 and 3.3 μmol of [^{3}H]water into DPS per hr, respectively (54.4 nmol/hr per g times 170 g divided by 1000 nmol/μmol and 468 nmol/hr per g times 7.1 g divided by 1000 nmol/μmol).[2] From the slopes of the linear regression curves in Fig. 5 and the mean hepatic content of [^{3}H]DPS in control animals, it can be further calculated that the carcass and small bowel took up from the blood per hr an amount of newly synthesized DPS containing about 1.2 and 0.4 μmol of [^{3}H]water, respectively (2300 nmol/hr per g times 0.003 times 170 g divided by 1000 nmol/μmol and 2300 nmol/hr per g times 0.025 times 7.1 g divided by 1000 nmol/μmol).[2] Thus, the total tissue content of [^{3}H]DPS in these experiments equalled 10.5 and 3.7 μmol/hr, respectively, in the carcass and small intestine. These values are nearly identical to the corresponding tissue contents that can be calculated from the data obtained in control animals and shown in Fig. 1.

The third question dealt with in this experiment concerned the possible transfer of newly synthesized [^{3}H]DPS from the intestine to the liver. Within the 1-hr interval between the administration of the [^{3}H]-water and the time the animals were killed, it was possible that significant amounts of sterol had been synthesized in the intestine, incorporated into chylomicrons and transported to the liver. Such a transfer would not have been detected by the appearance of significant amounts of [^{3}H]DPS in the blood even

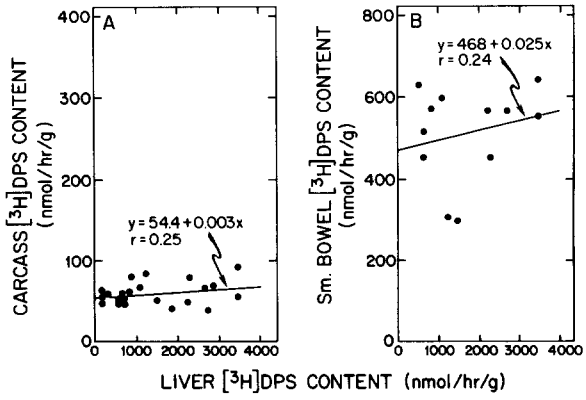


Fig. 5. Relationship between the amount of newly synthesized sterol in the carcass (Panel A) and small bowel (Panel B) and the [^{3}H]DPS content of the liver. These data were derived from the same group of animals described in the legend to Fig. 4.

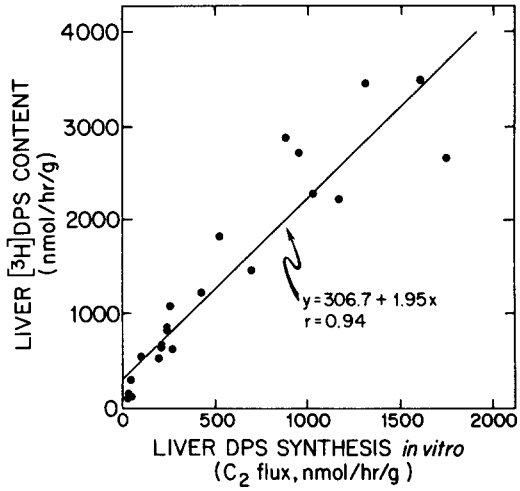


Fig. 6. Relationship between the content of [^{3}H]DPS in the liver in vivo and the rate of hepatic sterol synthesis measured in vitro. Utilizing the protocol described in the legend to Fig. 4, 22 animals were administered varying amounts of chylomicron cholesterol to cause varying degrees of suppression of the rate of hepatic sterol synthesis. Twelve hours later each animal was administered [^{3}H]water and 1 hr after the injection the content of [^{3}H]DPS in the liver was determined. Slices of the same liver were then incubated with [1-^{14}C]octanoate for determination of rates of hepatic sterol synthesis under in vitro conditions. In this diagram the content of [^{3}H]DPS found in the liver in vivo in each animal (vertical axis) is plotted against the rate of sterol synthesis measured in the same liver in vitro (horizontal axis).

when synthesis of the liver was fully suppressed (Fig. 4) since the remnant uptake process in the liver can achieve a high velocity at a very low concentration of remnant particles in the plasma (28). As a consequence, a considerable amount of [^{3}H]DPS could have passed through the blood compartment without significantly elevating the [^{3}H]DPS content of blood. In an attempt to quantitate the rate of such transfer, both the [^{3}H]DPS content and the rate of [^{14}C]DPS synthesis from [1-^{14}C]octanoate was measured in the livers of the group of animals administered varying amounts of chylomicrons. One hr after the administration of the [^{3}H]water, the rats were killed and aliquots of liver were taken for measurement of [^{3}H]-DPS content and for assay of rates of sterol synthesis under in vitro conditions. These data are shown in **Fig. 6**, where the content of [^{3}H]DPS found in vivo in each liver (vertical axis) is plotted against the rate of DPS synthesis measured in the same liver under in vitro conditions (horizontal axis). In theory, if the linear regression curve describing the relationship between these two values extrapolated to 0 [^{3}H]DPS content when the C_2 flux into DPS was reduced to 0, it would imply that there was no significant transfer of [^{3}H]DPS from the intestine to the liver under in vivo conditions and that all of the [^{3}H]DPS found in the liver was synthesized within that tissue. In fact,

TABLE 2. Corrected [^{3}H]DPS content in tissues of rats

Experimental Group	Measurement	1 Liver	2 Carcass	3 Skin	4 Small Bowel	5 Blood
Operated controls	A. Tissue [^{3}H]DPS content (nmol/hr/g)	2027 ± 128	52 ± 3	110 ± 16	540 ± 19	214 ± 9
	B. Residual blood volume in tissue (mg/g)	70 ± 6	8 ± 3	6 ± 1	12 ± 2	
	C. [^{3}H]DPS in residual blood in each tissue (nmol/hr/g)	15 ± 1	2 ± 0	1 ± 0	3 ± 1	
	D. Corrected [^{3}H]DPS tissue content (nmol/hr/g)	2164 ± 131	50 ± 3	109 ± 16	544 ± 21	214 ± 9
	E. Corrected [^{3}H]DPS tissue content (μmol/hr/organ)	16.01 ± 0.92	6.60 ± 0.41	4.03 ± 0.72	3.97 ± 0.25	2.74 ± 12
Small bowel removed	F. Corrected [^{3}H]DPS tissue content (nmol/hr/g)	1863 ± 31	40 ± 3	108 ± 12		116 ± 8
	G. Corrected [^{3}H]DPS tissue content (μmol/hr/organ)	13.87 ± 0.24	5.67 ± 0.44	4.09 ± 0.81		1.70 ± 0.11
Liver and small bowel removed	H. Corrected [^{3}H]DPS tissue content (nmol/hr/g)		36 ± 3	66 ± 9		58 ± 5
	I. Corrected [^{3}H]DPS tissue content (μmol/hr/organ)		5.19 ± 0.31	2.51 ± 0.40		0.75 ± 0.10

As described in the Materials and Methods section, the tissue content of [^{3}H]DPS was measured in operated control rats (line A) and in animals in which the small intestine or the small intestine plus about 90% of the liver had been removed. In parallel experiments the residual blood volumes in each organ were also measured (as shown for the control group in line B). Using these latter values, the amount of [^{3}H]DPS present in the whole blood contained in the organs was calculated (line C). The data in lines B and C were then used to correct the values in line A and yielded the values shown in line D. These numbers represent the nmol of [^{3}H]water incorporated into DPS per hr

as seen in Fig. 6, the linear regression curve had an intercept of 306.7 nmol/hr per g on the vertical axis. This intercept was significantly different from 0 at the $P < 0.05$ level. This finding suggested that under circumstances where the rate of hepatic cholesterol synthesis was suppressed to 0, [^{3}H]DPS still appeared in the liver. This labeled sterol presumably was synthesized in the intestine and transferred to the liver via chylomicrons. If this interpretation is correct then the minimal amount of [^{3}H]DPS transferred to the liver could be calculated to equal 2.21 μmol/hr (306.7 nmol/hr per g times 7.2 g divided by 1000 nmol/μmol).

The second model utilized in these studies to quantitate rates of sterol synthesis and transfer in vivo was to examine the effects of the acute surgical removal of the small intestine and liver on the content of newly synthesized DPS in the various tissues of the rat. In addition, in these particular experiments, the content of [^{3}H]DPS in each organ was also corrected for the [^{3}H]DPS contained in the small amount of residual blood retained within the tissues. The results of these studies are summarized in **Table 2.** The mean plasma cholesterol levels were the same in the control animals (64 ± 3 mg/dl) and in the rats subjected to enterectomy (67 ± 2 mg/dl) and to the surgical removal of both the liver and small bowel (63 ± 4 mg/dl). Line A shows the tissue content of [^{3}H]DPS in 1 g of each major organ 1 hr after administration of the [^{3}H]water to operated control animals. It should be noted that in column 2 the carcass contained all of the tissues of the animal not otherwise noted in the other columns. Line B gives the amount of residual blood retained in each tissue as determined using ^{51}Cr-labeled red blood cells. Using these values and the content of [^{3}H]DPS in the blood (column 5), the amount of [^{3}H]DPS in the residual blood in each organ could be calculated as given in line C. Utilizing the data in lines B and C, the corrected tissue contents of [^{3}H]DPS were obtained as listed in line D. When these values were multiplied by the organ weights, the values for the corrected [^{3}H]DPS content in the whole organs, as shown in line E, were obtained. As in the earlier study, the highest content of [^{3}H]DPS per g of tissue (line D) was found in the liver (2164 nmol/hr per g), adrenal gland

subjected to surgical removal of the small bowel and liver

6 Lung	7 Brain	8 Kidney	9 Spleen	10 Ovary	11 Adrenal	12 Heart	13 Whole Animal
179 ± 28	112 ± 6	68 ± 9	197 ± 30	581 ± 118	907 ± 125	37 ± 6	
131 ± 7	12 ± 2	84 ± 18	155 ± 28	31 ± 10	51 ± 2	80 ± 5	
28 ± 4	3 ± 1	18 ± 1	33 ± 4	7 ± 1	11 ± 2	17 ± 2	
174 ± 30	110 ± 6	55 ± 9	194 ± 32	592 ± 121	945 ± 130	22 ± 5	
0.24 ± 0.02	0.19 ± 0.01	0.09 ± 0.01	0.09 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.02 ± 0.00	34.10
138 ± 11	113 ± 8	51 ± 3	118 ± 10	624 ± 85	672 ± 123	25 ± 1	
0.15 ± 0.01	0.20 ± 0.02	0.09 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.04 ± 0.00	0.02 ± 0.00	25.95
90 ± 7	102 ± 6	31 ± 1	87 ± 7	570 ± 23	319 ± 53	14 ± 3	
0.14 ± 0.01	0.18 ± 0.02	0.06 ± 0.01	0.04 ± 0.00	0.05 ± 0.01	0.02 ± 0.00	0	8.94

by 1.0 g of each tissue after correcting for the [^{3}H]DPS present in blood contained in that tissue. These corrected values were also multiplied by the weights of each tissue to give the corrected amount of [^{3}H]DPS present in each organ (line E) and expressed as the μmol of [^{3}H]water incorporated into DPS per hr per organ. Identical measurements and corrections were made in the animals subjected to enterectomy and hepatectomy but, for the purpose of brevity, only the final corrected values are shown for these groups of animals (lines F, G, H, and I). The values represent means ± 1 SEM of data obtained in four animals in each group.

(945 nmol/hr per g), ovary (592 nmol/hr per g), and small bowel (544 nmol/hr per g). When these corrected values were expressed as the [^{3}H]DPS content per whole organ (line E), most of the newly synthesized sterol was present in the liver (16.01 μmol/hr), carcass (6.60 μmol/hr), skin (4.03 μmol/hr), small bowel (3.97 μmol/hr), and blood (2.74 μmol/hr). Thus, of the total amount of [^{3}H]DPS synthesized in the whole animal in 1 hr (34.10 μmol/hr), 47% was found in the liver, 19% in carcass (principally muscle, marrow, colon, and adipose tissue), 12% in skin, 12% in small bowel, and 8% in blood. It should be noted that both the relative distribution of [^{3}H]DPS in the various organs and the absolute rates of sterol synthesis found in these operated control animals were essentially identical to those values found in the unoperated control animals shown in Fig. 1. The surgical procedure apparently had no effect on either the rate of synthesis or the tissue distribution of [^{3}H]DPS under these experimental conditions.

When the small intestine was removed from the experimental animal, the rate of incorporation of [^{3}H]water into DPS in the whole animal decreased from 34.10 μmol/hr to 25.95 μmol/hr (line G) and when most of the liver was also removed the rate of sterol synthesis decreased further to 8.94 μmol/hr (Line I). From these values it could be calculated that the liver apparently accounted for an incorporation of 17.01 μmol/hr of [^{3}H]water into DPS while the small intestine and remaining tissues of the body accounted for sterol synthesis equivalent to 8.15 μmol/hr and 8.94 μmol/hr, respectively. Thus, of the total amount of [^{3}H]DPS synthesized in the whole animal (34.10 μmol/hr), 50% was synthesized in the liver, 24% in the small bowel, and 26% in the remaining tissues of the carcass.

When the small intestine was removed, the [^{3}H]-DPS content of 1 g of liver decreased from 2164 nmol/hr per g (line D) to 1863 nmol/hr per g (line F). This decrement in content of 301 nmol/hr per g was almost identical to the content of [^{3}H]DPS (306.7 nmol/hr per g) found in the liver in the preceeding experiment in animals where hepatic synthesis was suppressed to 0 by the administration of chylomicrons

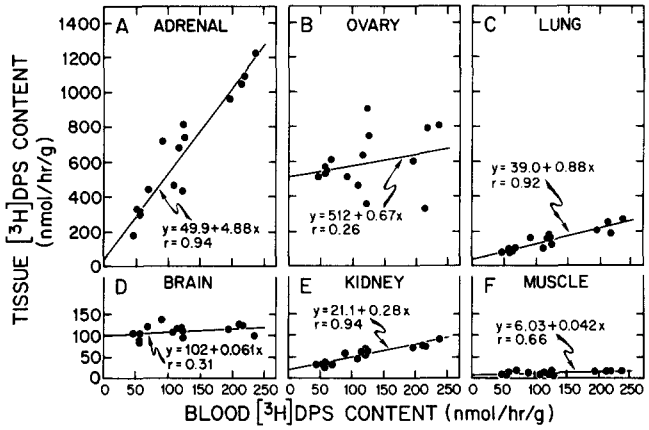


Fig. 7. Relationship between the [^{3}H]DPS content of six different tissues and the content of [^{3}H]DPS in the blood of the same animal. These data were obtained in rats subjected to surgical resection of the small intestine or small intestine and liver as described in the legend to Table 2 and the data have been corrected for contamination of the tissues with residual amounts of blood. In this diagram the content of [^{3}H]DPS in six different tissues is plotted against the content of [^{3}H]DPS in the blood of the same animal.

(Fig. 6). This finding again demonstrates that similar results were obtained in the operated and unoperated animal models and confirmed that about 2.1 μmol/hr of the [^{3}H]DPS content in the liver of control rats had been synthesized in the small intestine. It is also apparent from Table 2 that there were significant decreases in the content of [^{3}H]DPS in the blood and other organs following removal of the intestine (lines F and G) or intestine and liver (lines H and I). This finding also is consistent with the results obtained in the preceding group of studies indicating that there was uptake of [^{3}H]DPS from the blood by the peripheral tissues of the carcass and that nearly all of the [^{3}H]DPS in the blood was derived directly from sterol synthesis in the liver or indirectly from sterol synthesis in the intestine (after transport to the liver). However, it is also apparent that there were marked differences in the dependency of the [^{3}H]-DPS content of different organs on sterol synthesis in the liver and gut. For example, in brain there was essentially no effect on the content of [^{3}H]DPS when the animals were eviscerated (column 7) while the content of [^{3}H]DPS in the adrenal gland decreased markedly (column 11). The other organs manifested intermediate effects of this type. This finding implied that some organs, like the brain, relied principally upon de novo synthesis of sterol to meet their cholesterol needs while other organs, such as the adrenal gland, relied principally on uptake from the blood of cholesterol synthesized remotely in the liver and small bowel.

Utilizing these same data, it was possible to determine in detail the rate of sterol synthesis and the rate of sterol uptake in each organ. As shown in **Fig. 7** for six different tissues, the [^{3}H]DPS content of each organ was plotted against the [^{3}H]DPS content of blood found in the same animal: these 15 animals were derived from the three experimental groups described in Table 2. In theory, the intercept of the linear regression curve on the vertical axis, where the [^{3}H]-DPS content of blood equalled zero, should give the rate of sterol synthesis in a particular organ while the slope of the regression curve should equal the rate of tissue uptake of [^{3}H]DPS from the blood. Thus, the adrenal gland, for example, must have a low rate of synthesis (49.9 nmol/hr per g) but a very high rate of uptake from the blood (4.88 nmol/hr per g per 1.0 nmol/hr per g of [^{3}H]DPS in blood). In contrast, brain has a rate of synthesis equal to 102 nmol/hr per g but takes up virtually no [^{3}H]DPS sterol from the blood (0.061 nmol/hr per g per 1.0 nmol/hr per g).

Based on this type of analysis and on the data presented in Table 2, the rates of sterol synthesis in the major tissues of the rat in vivo were as follows: liver, 2,363 nmol/hr per g; small intestine 1,150; ovary, 512; brain, 102; skin, 53; adrenal gland, 50; lung, 39; carcass (principally muscle, bone marrow, and colon), 33; adipose tissue, 25; kidney 21; spleen, 14; heart, 12; and striated muscle, 6. Similarly, the rates of uptake of [^{3}H]DPS from the blood were as follows: adrenal gland, 4.88 nmol/hr per g per 1.0 nmol/hr per g of blood [^{3}H]DPS; spleen, 1.10, lung, 0.88; ovary, 0.67; skin, 0.34; kidney, 0.28; small intestine, 0.26; heart, 0.15; carcass, 0.063; brain, 0.061; muscle, 0.042; and adipose tissue, 0.039. It was not possible from these experiments to quantitate the rate of hepatic uptake of [^{3}H]DPS from the blood. Thus, the content of the [^{3}H]DPS in the various tissues, as shown in Fig. 1 and Table 2, reflect the sum of the [^{3}H]DPS synthesized in each tissue and the amount of [^{3}H]DPS taken up by that tissue from the blood (or, in the case of the small intestine, lost to the liver).

In a final group of studies an attempt was made to determine if the rates of synthesis measured in each organ in vivo were regulated by circulating levels of lipoprotein cholesterol. As we have previously described in detail (20), when animals were treated for 4 days with APP the plasma cholesterol levels dropped from about 60 mg/dl to <5 mg/dl. Because such animals ate poorly, all rats were fasted for this 4-day experiment and infused with a hypertonic glucose solution for 12–14 hr prior to the administration of [^{3}H]water. As shown in **Table 3**, the control rats incorporated a total of 17.43 μmol/hr of [^{3}H]water

into DPS. This reduced rate of whole animal synthesis reflected the partially-fasted state of these animals. Many of the individual organs also showed a reduced content of [^{3}H]DPS (lines A or B, Table 3) when compared to the content of [^{3}H]DPS in the same organs of fully-fed control animals (lines D or E, Table 2). In the animals treated with APP, whole animal sterol synthesis equalled only 11.68 μmol/hr (line D). However, it is apparent that the lower rate of whole-animal synthesis was the result of the hepatotoxic effect of APP and the consequent marked reduction in the content of [^{3}H]DPS in the liver from 1510 nmol/hr per g in the control animals to only 120 nmol/hr per g in those animals administered APP. In contrast to this effect in the liver, however, the content of [^{3}H]DPS in tissues such as duodenum, jejunum, ileum, colon, kidney, ovary, and adrenal gland was actually significantly higher in the APP-treated rats than in the control animals.

It should be noted that because the blood content of [^{3}H]DPS is much higher in the control animals than in those treated with APP, there was greater uptake of [^{3}H]DPS from the blood by the organs of the control rats. This would tend to lessen and obscure the differences in rates of de novo synthesis that occurred in the tissues of these two groups of animals. If one assumes that the rate constants for uptake determined in the preceding experiment (Fig. 7) apply to these animals, then mathematically one can extrapolate the content of [^{3}H]DPS in each tissue to the level it should equal if the content of [^{3}H]DPS in the blood equalled zero. When this is done, for example, the actual rates of synthesis in the control and APP-treated animals, respectively, equal 24 and 55 nmol/hr per g in skin, 186 and 748 nmol/hr per g in the jejunum, 35 and 112 nmol/hr per g in the lung, 7 and 84 nmol/hr per g in the kidney, 42 and 205 nmol/hr per g in spleen, 1556 and 3841 nmol/hr per g in the ovary, and 911 and 2794 nmol/hr per g in the adrenal gland. In contrast, there were no differences in the rates of synthesis in brain, heart, or striated muscle. Thus, it is apparent that markedly lowering the circulating levels of plasma cholesterol with APP caused a 2.3- to 12-fold increase in the rate of sterol synthesis by tissues such as bowel, kidney, the endocrine glands, and lung, but had no effect on muscle or brain.

DISCUSSION

These studies were designed to quantitate the rates of sterol synthesis and uptake in all of the major tissues of the rat under in vivo conditions. In order to dissect the specific contributions of the major tissues to total body sterol synthesis, it was necessary to utilize three different animal models: *1*) control rats that had not been subjected to any manipulation (Figs. 1 and 2); *2*) animals in which hepatic sterol synthesis had been selectively inhibited by chylomicron administration (Figs. 3–6); and *3*) animals in which either the small bowel or liver and small bowel had been surgically removed (Fig. 7 and Table 2). Obviously, it is important to determine that these two latter experimental manipulations did not themselves alter rates of sterol synthesis in the major extrahepatic tissues. There are a number of comparisons that can be made that suggest that this was the case. First, the content of [^{3}H]DPS in the liver, small bowel, and blood of the unoperated (Fig. 1) and operated (Table 2) control animals was essentially identical. Furthermore, the distribution of [^{3}H]DPS in the other tissues of the carcass was very similar. Second, the content of [^{3}H]DPS in the carcass (all tissues other than liver, small intestine, and blood) equalled 10.5 μmol/hr in the animals administered chylomicrons (Fig. 5) and 11.4 μmol/hr in the animals subjected to surgery (Table 2). Third, total body synthesis equalled 31.5 μmol/hr in the animals not subjected to any experimental manipulation and 34.10 μmol/hr in the operated animals. Fourth, the rate of synthesis of sterols in the combined tissues of the carcass (all organs other than the liver and small intestine) was calculated to equal 9.3 μmol/hr in the chylomicron infused rats (Fig. 5) and was directly determined to equal 8.9 μmol/hr after surgical removal of the liver and small intestine (Table 2). Finally, about 2.2 μmol/hr of [^{3}H]-DPS was found in the liver under circumstances where hepatic sterol synthesis had been suppressed to 0 by chylomicron administration (Fig. 6), and the content of [^{3}H]DPS in the liver was reduced by 2.1 μmol/hr when the small intestine was surgically removed (Table 2). Thus, taken together, the excellent agreement among these different measurements in the three experimental groups of animals strongly suggests that neither the surgical removal of the small bowel or liver nor the selective inhibition of hepatic sterol synthesis by chylomicron infusion artifactually altered rates of sterol synthesis or uptake in the remaining tissues of the body.

Assuming then, that the data obtained in all three animal models are essentially comparable, it is possible to draw several major conclusions concerning the rates of sterol synthesis and uptake in the major tissues of the rat under in vivo conditions. The first point concerns the distribution of synthetic activity in the four major tissue compartments, i.e., the liver, small bowel,

TABLE 3. Corrected [^{3}H]DPS content in tissues of rats treated

Measurement	1 Liver	2 Carcass	3 Skin	4 Stomach	5 Duodenum	6 Jejunum	7 Ileum	8 Colon
A. Corrected [^{3}H]DPS tissue content (nmol/hr/g)	1510 ± 275	26 ± 4	57 ± 2	196 ± 12	315 ± 23	187 ± 18	236 ± 46	102 ± 16
B. Corrected [^{3}H]DPS tissue content (μmol/hr/organ)	9.47 ± 1.46	3.10 ± 0.45	1.80 ± 0.15	0.26 ± 0.05	0.28 ± 0.04	0.39 ± 0.03	0.26 ± 0.07	0.80 ± 0.00
C. Corrected [^{3}H]DPS tissue content (nmol/hr/g)	120 ± 16	22 ± 1	60 ± 4	252 ± 55	746 ± 41	574 ± 27	930 ± 12	570 ± 6
D. Corrected [^{3}H]DPS tissue content (μmol/hr/organ)	1.15 ± 0.18	2.84 ± 0.34	2.10 ± 0.14	0.30 ± 0.06	0.83 ± 0.14	1.37 ± 0.17	1.04 ± 0.13	0.54 ± 0.11

As described in the Materials and Methods section, fasted rats were treated with APP for 4 days to lower the circulating levels of plasma cholesterol to <5 mg/dl. The animals were then administered [^{3}H]water and the content of [^{3}H]DPS was determined in a number of tissues. These values were corrected for the amount of [^{3}H]DPS present in whole blood still contaminating the tissues as described in Table 2. Only the corrected values, however, are shown in this table for the control (lines A and B) and drug-treated (lines C and D) groups of rats. The values represent means ±1 SEM for data obtained in four animals in each group.

blood, and remaining tissues of the carcass, and is shown in diagrammatic form in **Fig. 8.** In the fed animal weighing about 200 g and studied at the mid-dark point of the light cycle, total body synthesis is equal to the incorporation of approximately 34.1 μmol of [^{3}H]water into DPS per hr (Table 2). Of this total rate of synthesis, about 17.0 μmol/hr occurred in the liver (50%), 8.2 μmol/hr took place in the small bowel (24%), and the remaining 8.9 μmol/hr was made in the tissues of the carcass (26%) (Table 2). One hour after administration of the [^{3}H]water, the content of [^{3}H]DPS in these three tissues equalled 16.0 μmol/hr (liver), 4.0 μmol/hr (small bowel), and 11.4 μmol/hr (carcass) (Table 2). In addition, the blood had acquired a [^{3}H]DPS content equivalent to 2.7 μmol/hr (Fig. 1, Table 2). As also shown in Fig. 8, there were significant shifts of newly synthesized sterol between the major tissue compartments. During the 1-hr period of observation, the small bowel took up about 0.4 μmol of [^{3}H]DPS from the blood (Fig. 5). Since the content of [^{3}H]DPS in the intestine was 4.0 μmol/hr and this organ had acquired a total of 8.6 μmol/hr through synthesis and uptake from the blood, it follows that 4.6 μmol/hr of [^{3}H]DPS left the bowel and was transported to the liver, presumably carried in chylomicrons. This amount was added to the 17.0 μmol/hr synthesized within the liver. Again, since the content of the liver was 16.0 μmol/hr, 5.6 μmol/hr must have left the liver and entered the blood compartment. Of this amount, 0.4 μmol/hr was removed by the intestine and 2.5 μmol/hr was taken up by the various tissues of the carcass leaving behind 2.7 μmol/hr in the blood. The tissues of the carcass synthesized an additional 8.9 μmol/hr giving a total content of 11.4 μmol/hr.

It is apparent that this diagram shows an essentially unidirectional flow of newly synthesized cholesterol from the liver (and, indirectly, from the small bowel) to the blood and, ultimately, to the peripheral tissues of the carcass. In the steady-state, there must be an equal but oppositely directed flux of cholesterol from the carcass back to the liver, the major site for sterol excretion. However, since the specific activity of cholesterol in the peripheral tissues is so low (Fig. 2), this back flux does not cause any significant underestimation of the calculated rates of transfer. Indeed, as shown in Fig. 4, when synthesis in the liver is totally suppressed, a maximum of only about 0.09 μmol/hr of newly synthesized sterol could have entered the blood from the carcass and/or small bowel. Thus, because of the relatively short period of observation that was deliberately utilized in these studies, the tissues of the carcass can be considered as an infinite sump for the uptake of [^{3}H]DPS from the blood.

From this analysis, then, the tissues of the carcass synthesized a total of 8.9 μmol/hr of [^{3}H]DPS and took up only about 2.5 μmol/hr from the blood. Using data derived from an independent analysis of the relationship between synthesis and uptake in each of the major tissues of the carcass (Fig. 7), it is possible to describe further the events taking place in the individual organs of this compartment. As outlined in

with APP to lower circulating levels of lipoprotein cholesterol

9 Blood	10 Lung	11 Brain	12 Kidney	13 Spleen	14 Ovary	15 Adrenal	16 Heart	17 Muscle	18 Whole Animal
96 ± 15	119 ± 8	60 ± 3	34 ± 3	148 ± 14	1620 ± 491	1379 ± 292	15 ± 3	6 ± 1	
1.14 ± 0.05	0.12 ± 0.01	0.11 ± 0.01	0.06 ± 0.01	0.09 ± 0.00	0.13 ± 0.04	0.12 ± 0.03	0.02 ± 0.00		17.43
16 ± 2	126 ± 10	64 ± 5	89 ± 4	222 ± 36	3851 ± 1063	2872 ± 737	12 ± 2	7 ± 2	
0.20 ± 0.02	0.16 ± 0.02	0.12 ± 0.01	0.15 ± 0.01	0.11 ± 0.02	0.33 ± 0.09	0.43 ± 0.11	0.01 ± 0.00		11.68

Fig. 9, the major portion of the [^{3}H]DPS synthesis detected in the carcass took place in the brain (0.19 μmol/hr), skin (2.65 μmol/hr), and muscle and bone marrow combined (5.28 μmol/hr). In these organs, therefore, 76–89% of the tissue content of [^{3}H]DPS came from de novo synthesis. In contrast, most of the [^{3}H]DPS in organs such as the adrenal gland, spleen, kidney, and lung was taken up from the blood and, therefore, these tissues contributed little to sterol synthesis in the carcass. It should be noted that the sums of the rates of uptake and synthesis in these individual organs, i.e., 2.7 μmol/hr and 8.3 μmol/hr, respectively, are very close to the corresponding values determined for the whole carcass in the animals subjected to surgery or chylomicron infusion and provide further support for the validity of these various experimental models.

In theory, there are probably two separate mechanisms that could account for the movement of newly synthesized sterol between the major tissue compartments. First, the [^{3}H]DPS could be incorporated into specific lipoproteins and secreted into the blood, e.g., as occurs in the intestine and liver, and the same lipoproteins, in turn, could then be taken up by other tissues, e.g., the liver, adrenal gland, kidney, etc. Second, there could also be movement of molecular [^{3}H]cholesterol from the various tissues to lipoproteins in the plasma followed by movement of this labeled sterol from the lipoproteins into other organs. The actual mechanism of this "exchange" reaction is poorly understood but the most likely explanation is that it takes place through a small, but finite, concentration of molecular cholesterol in solution in the aqueous phase of plasma that is in equilibrium with unesterified cholesterol in the various tissues and lipoproteins. If this is the case, then the magnitude of such unidirectional uptake of unesterified [^{3}H]DPS from the blood to a particular tissue would be determined by the product of the concentration of cholesterol in solution in equilibrium with the lipoproteins and the passive permeability coefficient for the cholesterol molecule in the cell membrane of the target tissue (29–32). In the present studies it was of considerable interest that there was very little equilibration of [^{3}H]DPS between the plasma and most of the peripheral tissues. For example, the specific activity of unesterified cholesterol in most tissues was <30% of the specific activity of the plasma cholesterol and in major tissues such as muscle, kidney and brain the specific activity was <3% of that of plasma. Furthermore, as discussed below, there is good correlation between the rate of lipoprotein transport known to occur in many tissues of the carcass and the rate of [^{3}H]DPS uptake observed in the present studies. Thus, while we made no attempt to identify the mechanism(s) responsible for the uptake of newly synthesized sterols, it is likely that the rates of uptake shown in Figs. 8 and 9 reflect primarily lipoprotein transport and not monomolecular cholesterol diffusion. This is not suprising in view of the fact that the passive permeability coefficient for cholesterol in many cell membranes and its aqueous solubility are

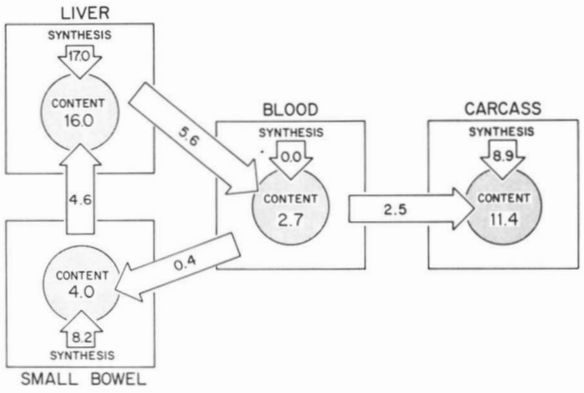
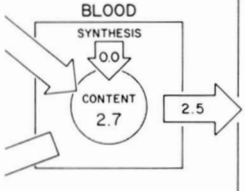


Fig. 8. Diagrammatic representation of the sites of sterol synthesis and the rates of sterol movement between major tissue compartments in the fed rat weighing about 200 g at the mid-dark phase of the light cycle. In this diagram the whole body has been divided into four major compartments: the liver, small bowel, blood, and remaining tissues of the carcass. Three sets of data are shown for each tissue compartment: the amount of sterol that is synthesized de novo in that organ, the amount of newly synthesized DPS that moves out of the tissue, and the content of newly synthesized DPS that remains in the tissue. These values are given in terms of the amount of sterol that is synthesized de novo per hr and are expressed as the μmol of [^{3}H]water incorporated into DPS per hr. Assuming that 1.45 C atoms are incorporated into DPS for each ^{3}H atom (13), then these values may be multiplied by the factor 0.0312 to yield rates of synthesis and transfer that have the units of mg of cholesterol synthesized per hr. For example, the rate of sterol synthesis in the liver equals 17.0 μmol of [^{3}H]water incorporated into DPS per hr which is equivalent to the synthesis of 0.53 mg of cholesterol per hr. The manner in which these various rates were calculated is described in detail in the discussion.

both low and would limit the magnitude of the unidirectional flux of cholesterol from plasma to the cells of specific organs.[3] In most published investigations in which cholesterol "exchange" has been considered to be quantitatively important, the experiments were carried out over many days, weeks, or even months.

The data obtained in this study on the quantitative aspects of sterol synthesis in vivo can be compared to the extensive information available in the same species when rates of synthesis are measured utilizing in vitro techniques. Three major points warrant emphasis. The first deals with the quantitative importance of the liver and intestine, relative to the remaining tissues of the carcass, as sites for sterol synthesis in the whole animal. Utilizing data obtained by measuring rates of incorporation of [^{14}C]acetate into DPS in tissue slices, it has been reported that fully 90% of all detectable synthetic activity measured under such in vitro conditions takes place in the liver and small intestine (2). With the recognition that there is poor uptake or activation of acetate or disproportionate dilution of the specific activity of the intracellular acetyl CoA pool in many of the nonhepatic, nonintestinal tissues (13), it is likely that the importance of these tissues to total body sterol synthesis has been underestimated. Clearly, this prediction is supported by the results of the present study in which the tissues of the carcass were found to account for about 27% of the DPS synthesis occurring in vivo in the rat while the liver and intestine accounted for about 50% and 24%, respectively. Furthermore, when the rat is studied at the mid-light point of the light cycle where hepatic synthesis is significantly less, the small intestine and carcass become the predominant sources for whole body sterol synthesis (16).

The second point concerns the relative rates of sterol synthesis in each of the major tissues of the body when expressed per unit weight. In in vitro studies, the liver and small bowel have generally been reported to have the highest rates of sterol synthesis (2–4, 20, 27). Rates of acetate incorporation into DPS by most of the other tissues of the body are much lower and usually equal only about 1–2% of the rates observed in the liver. The lowest activities are routinely found in muscle (striated, smooth, or cardiac) and brain. The rates of synthesis measured in vivo with [^{3}H]water show the same general pattern of synthetic activity but the nonhepatic, nonintestinal tissues have rates of synthesis that are relatively higher equalling, for example, from 2–20% of the activity found in the liver. The brain is the one major exception to this good correlation between data obtained in vitro and in vivo.



CARCASS IN DETAIL

TISSUE	UPTAKE	SYNTHESIS	CONTENT
Adrenal	0.069	0.003 (4%)	0.072
Spleen	0.115	0.007 (6%)	0.122
Heart	0.023	0.009 (28%)	0.032
Kidney	0.110	0.039 (26%)	0.149
Lung	0.213	0.044 (17%)	0.257
Ovary	0.017	0.061 (78%)	0.078
Brain	0.024	0.19 (89%)	0.214
Skin	0.55	2.65 (83%)	3.20
Remaining Tissues, e.g., Muscle, Marrow	1.62	5.28 (76%)	6.90
TOTALS	2.7	8.3	11.0

Fig. 9. Diagrammatic representation of the rates of sterol synthesis and uptake by the major organs of the carcass. This diagram is a continuation of the one shown in Fig. 8 but shows the sites of tissue synthesis in more detail in the carcass compartment. The first column shows the rates of uptake of [^{3}H]DPS from the blood by each organ while the second column shows the rates of sterol synthesis in each tissue. The content of [^{3}H]DPS in each organ equals the sum of these two values and is given in the third column. The percentage of the content that is due to local synthesis is also shown in the second column.

[3] The passive permeability coefficient for a compound is principally determined by the polar characteristics of the membrane through which the solute must pass. Recent work has demonstrated that many biological membranes behave as relatively polar structures; hence, very nonpolar molecules such as cholesterol have lower passive permeability coefficients than might be anticipated (31).

In in vitro studies it is found that virtually no ^{14}C-labeled substrate such as acetate, glucose, or octanoate is incorporated into DPS by tissues from the central nervous system. In contrast, in the present study, the cerebrum had a rate of synthesis that equalled 102 nmol/hr per g, which was higher than any tissue of the body with the exception of the liver, intestine, and ovary. Presumably under in vitro conditions the ^{14}C-labeled substrates either cannot penetrate the various diffusion barriers to reach the actively synthesizing brain cells or are not metabolized intracellularly to a usable substrate for sterol synthesis. In these in vivo studies, [^{3}H]water presumably is delivered directly to the cells of the central nervous system through the capillary beds, and rapidly penetrates the blood–brain barrier to be incorporated into newly synthesized sterols. It seems likely, therefore, that rates of sterol synthesis in brain have been markedly underestimated in most previously reported studies. It should be noted, however, that the rat is a growing animal. Whether such high rates of sterol synthesis would be found in the mature, non-growing brain of another animal species is not known.

The third major point that derives from the present studies, as well as from a previous publication from this laboratory (16), concerns the mechanisms that act to regulate sterol synthesis in the major organ systems of the body under in vivo conditions. Many such mechanisms have been identified and characterized using various in vitro techniques to assay rates of sterol synthesis or levels of 3-hydroxy-3-methylglutaryl CoA reductase in specific organs (1). However, in recent publications it has been suggested that several of these mechanisms may not actually operate to regulate sterol synthesis under in vivo conditions (33, 34). Our studies do not support this point of view. Utilizing the [^{3}H]water method, it has been shown in the rat in vivo that liver manifests a diurnal variation in the rate of synthesis (16); furthermore, hepatic sterol synthesis is suppressed by cholesterol feeding, by administration of chylomicrons, and by fasting, and is markedly enhanced by stress or by interruption of the enterohepatic circulation of bile acids (16). These changes in rates of hepatic synthesis measured in vivo are in the same direction and of approximately the same order of magnitude as has been reported in studies using in vitro techniques (7, 20, 27). Similarly, there is good agreement concerning regulation of sterol synthesis in the nonhepatic organs. In vitro studies have shown that synthetic activity in such tissues does not manifest diurnal variation and is not inhibited by cholesterol feeding or chylomicron administration (27): synthesis is, however, partially inhibited by fasting (27). Essentially identical results are evident in the in vivo studies utilizing [^{3}H]water to assess rates of sterol synthesis (16).

Another very important regulatory mechanism that has been identified utilizing in vitro assay procedures is that mediated through the cellular uptake of cholesterol carried in low density lipoproteins (35, 36). By this mechanism cholesterol is transported from the liver to a number of organs in the periphery where it is taken up through a specific LDL receptor mechanism (37, 38). In addition, in the rat, cholesterol carried in high density lipoproteins also is apparently transported into the cells of the adrenal gland, ovary, and testes (21, 39). Cholesterol derived from these lipoprotein sources not only supplies the metabolic needs of these various tissues for sterol but, in addition, partially suppresses rates of de novo synthesis in the target organs. Thus, when the circulating levels of lipoprotein cholesterol are markedly suppressed by administration of drugs such as APP, there is an increase in rates of sterol synthesis (20, 40, 41). The present study confirms this finding in vivo and provides direct quantitation of the relative amount of plasma cholesterol taken up in the various tissues of the carcass. It is of particular interest that the group of tissues identified in vivo in which rates of sterol synthesis are responsive to circulating levels of lipoproteins, e.g., adrenal gland, lung, kidney, etc., include essentially the same organs as have been previously identified utilizing in vitro assay procedures (20). Furthermore, rates of synthesis in tissues like muscle and brain do not respond to fluctuations in plasma cholesterol levels under either in vivo or in vitro conditions. Thus, it is apparent that every major regulatory mechanism that has been identified utilizing in vitro techniques can be shown to operate in vivo in the intact animal.

Finally, the question can be raised as to the applicability of these data to higher animals and, particularly, to man. In order to answer this question a detailed comparison is currently underway in this laboratory of the sites of sterol synthesis in vivo in a number of other species. While these data will be published elsewhere, it can be stated that the distribution of synthetic activity in one primate, the squirrel monkey, is essentially identical to that reported here for the rat. Other species such as the rabbit, hamster, and guinea pig, however, have different patterns with respect to the major sites for tissue sterol synthesis.

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