Sterol synthesis in vivo in 18 tissues of the squirrel monkey, guinea pig, rabbit, hamster, and rat

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Abstract This study was undertaken to measure and compare the rates at which digitonin-precipitable sterols (DPS) were synthesized in vivo in the major organs of five different animal species. These rates were assessed by measuring the velocity at which [^{3}H]water was incorporated into DPS in the intact animal. The animals used were chosen to include species that carried most of their plasma cholesterol either predominantly in high (rat, hamster) or low (guinea pig) density lipoproteins (HDL and LDL, respectively) or more evenly distributed between the LDL and HDL fractions (monkey and rabbit). Whole animal sterol synthesis was much higher in the rat (16.1 μmol/hr) than in the other four species (2.9–4.6 μmol/hr) when normalized to a constant body weight of 100 g. This uniquely high rate of sterol synthesis could be attributed predominantly to an extremely high rate of incorporation of [^{3}H]water into DPS by the liver of the rat. When expressed per g of tissue, the highest content of newly synthesized sterol in all species was found in tissues such as adrenal gland, ovary, and gastrointestinal tract. However, the content of [^{3}H]DPS in the liver varied markedly from a high of 2279 nmol/hr per g in the rat to a low of only 109 nmol/hr per g in the guinea pig. Consequently, when expressed as a percentage of total body synthesis, the whole liver of the rat contained 51% of the [^{3}H]DPS while this figure was much lower in the monkey (40%), hamster (27%), rabbit (18%), and guinea pig (16%). Thus, in all species except the rat, the major sites for sterol synthesis appeared to be the gastrointestinal tract, carcass (predominantly the muscle), and skin. In addition, even though the content of newly synthesized sterol per g of adrenal gland was higher than in nearly any other tissue in all of the species examined, it was further demonstrated that in the rat most of this [^{3}H]DPS was derived from the blood (and, therefore, ultimately from the liver) whereas in the other species it was largely synthesized locally within the gland. Thus, these studies demonstrated that in many species the liver is quantitatively far less important as a site for sterol synthesis than previously believed and, as a correlate of this, most sterol utilized by extrahepatic tissues is largely synthesized locally within those tissues.—**Spady, D. K., and J. M. Dietschy.** Sterol synthesis in vivo in 18 tissues of the squirrel monkey, guinea pig, rabbit, hamster, and rat. *J. Lipid Res.* 1983. **24:** 303–315.

Supplementary key words cholesterol synthesis • [^{3}H]water • lipoprotein transport • liver

It is now generally believed that the various tissues of the body can acquire their necessary supply of cholesterol either by synthesizing it locally from acetyl CoA or by taking it up from the blood in such lipoproteins as chlomicrons (CM), very low density lipoproteins (VLDL), low density lipoproteins (LDL), or high density lipoproteins (HDL) (1–8). The extent to which a given tissue is dependent upon these two sources for cholesterol is dictated by the rate at which that tissue synthesizes cholesterol relative to the rate at which it takes up preformed sterol carried in the various lipoproteins. While there is currently considerable interest in the relationship that exists in different organ systems between these two processes, there is little quantitative data available on either the rates of lipoprotein uptake or the rates of cholesterol synthesis in specific organs under in vivo conditions (9–13). Furthermore, despite a number of years of work, it is still uncertain which of the organs of the body are important contributors to whole body sterol synthesis and whether such organs play the same quantitative roles in synthesizing cholesterol in different animal species (14). These questions are of considerable importance since they bear directly on the selection of animal models for studying problems related to lipoprotein metabolism and regulation of cholesterol balance in whole animals, and to the regulation of hepatic cholesterol secretion and cholesterol gallstone formation.

There are a number of technical reasons why uncertainty still exists as to the quantitative importance of different organs in whole-body sterol synthesis in animals and in man. First, external sterol balance techniques can quantitate the rate of cholesterol synthesis in the whole animal and in man but are nearly useless in dissecting out the quantitative importance of each organ system (15–17). Second, measurement of levels of 3-hydroxy-3-methylglutaryl CoA reductase (HMG CoA reductase) cannot be used for quantitating and

Abbreviations: DPS, digitonin-precipitable sterols; SA, specific activity; CM, chylomicrons; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; HMG, 3-hydroxy-3-methylglutaryl; C, carbon.

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comparing absolute rates of sterol synthesis in different organs since the conditions necessary for quantitative recovery of microsomes and for measuring enzymatic activity have been established in only a few tissues. Furthermore, it is not clear at this time whether it is the fluoride- or phosphatase-treated microsomes that give HMG CoA reductase activities that reflect the absolute rate of sterol synthesis in the whole organs (18). Third, measurement of rates of incorporation of ^{14}C-labeled substrates into cholesterol by whole cells or by tissue slices, while useful in defining relative rates of synthesis in the same tissue, give misleading data when used to compare rates of synthesis in different tissues. This difficulty arises from differences that exist in various tissues in the rate of cellular uptake and metabolism of the ^{14}C-labeled substrates and from marked differences that occur in the degree of intracellular dilution of the specific activity (SA) of the acetyl CoA pool derived from these substrates. In general, such substrates are more poorly utilized or the SA of the acetyl CoA pool undergoes much greater dilution in nonhepatic tissues than in the liver so that the importance of the liver to whole-body sterol synthesis has been systematically overestimated (19). Finally, with the possible exception of the isolated perfused liver, rates of sterol synthesis measured in vitro with almost any radiolabeled precursor grossly underestimate the absolute rates of synthesis that take place in the same tissues under in vivo conditions, even when corrections are made for dilution of the SA of the acetyl CoA pool (12).

In order to circumvent many of these problems, several laboratories have begun to quantitate rates of cholesterol synthesis by measuring the rates at which [^{3}H]water is incorporated into the sterol molecule (12, 13, 19–21). Not only can this precursor be utilized in vitro, but under in vivo conditions it appears to give absolute rates of sterol synthesis that are essentially equal to rates of whole-body sterol synthesis determined by external sterol balance techniques (17). This precursor has several major advantages over ^{14}C-labeled precursors. First, [^{3}H]water rapidly penetrates cell membranes and attains a uniform SA in the extra- and intracellular fluids (12). Second, since only very small amounts of unlabeled water are generated in biological systems, there is essentially no dilution of the SA of the intracellular pool of water. Third, the ^{3}H of the [^{3}H]water is incorporated into the sterol molecule either directly or after equilibration with the reductive H of NADPH and so, unlike the various ^{14}C-labeled precursors, does not have to be metabolized through a variety of enzymatic steps and pools of intermediate products (19, 20). There are, however, two important disadvantages to the use of [^{3}H]water. First, relatively large amounts of ^{3}H must be used because of the large size of the body pool of water and, second, calculation of absolute rates of cholesterol synthesis requires information of the μgatoms of ^{3}H incorporated into each μmol of sterol (19).

Utilizing [^{3}H]water, we have recently published detailed analyses of the quantitative importance of the major tissues of the rat to whole-animal sterol synthesis and have identified the regulatory mechanisms that are active in these tissues under in vivo conditions (12, 13). The present studies were undertaken using this same technique to quantitate and characterize sterol synthesis in vivo in the organs of other species such as the squirrel monkey, hamster, rabbit, and guinea pig that are commonly employed for studying cholesterol balance, lipoprotein metabolism, and biliary cholesterol secretion. These investigations point up very significant quantitative differences in cholesterol metabolism in these species and provide a rational basis for the selection of the best animal models to utilize in studying specific features of lipid and lipoprotein metabolism.

MATERIALS AND METHODS

Experimental animals

The female, Sprague-Dawley-derived rats (Charles River Breeding Laboratories, Wilmington, MA) weighed 150–175 g when received in the laboratory and were housed in colony cages with free access to water and Wayne Lab-Blox rat chow (Allied Mills, Inc., Chicago, IL) prior to use. The female, squirrel monkeys (South American Primates, Inc., Miami, FL) weighed 500–750 g when received and were housed individually with free access to water and Wayne Monkey Chow prior to use. The female, Golden Syrian-derived hamsters (Charles River Breeding Laboratories, Wilmington, MA) weighed 110–130 g when received and were housed in colony cages with free access to water and Wayne Lab-Blox. The rabbits used were females of the New Zealand White Strain (Sunny Acres Rabbitry, Tyler, TX) and were housed individually with free access to water and Wayne Rabbit Ration. The female guinea pigs of the Hartley strain (Dutchland Laboratory, Denver, CO) weighed 300–350 g on arrival and were housed in colony cages with free access to water and Wayne Guinea Pig Diet. All animals, except for the monkeys, were housed for at least 2 weeks before use in rooms with alternating periods of light (1500–0300 hr) and dark (0300–1500 hr). The monkeys were quarantined and treated for 6 weeks prior to use, as previously described (22). By direct analysis (by gas–liquid chromatography) the content of cholesterol in these various diets was as follows: Wayne Lab-Blox (0.26 mg/g), Wayne Monkey

diet (0.17 mg/g), Wayne Rabbit ration (0.011 mg/g), and Wayne Guinea Pig diet (0.011 mg/g).

Determination of sterol synthesis in vivo

As described previously (12, 13), the animals were administered [^{3}H]water (New England Nuclear, Boston, MA) intravenously as a bolus (approximately 200 to 500 mCi per kg body weight) in 0.5 to 1.0 ml of isotonic saline. In the studies with rats the animals were fitted with tail-vein catheters and placed in restraining cages. After the administration of [^{3}H]water, the catheters were immediately removed and the animals remained in the restraining cages until killed 1 hr later. In the experiments with squirrel monkeys and hamsters, the [^{3}H]water was administered directly into a femoral vein, guinea pigs were given the [^{3}H]water through the jugular vein, and the lateral ear vein was utilized in the rabbits. Following the injection of the [^{3}H]water, the animals were returned to individual cages under the fume hoods until killed 1 hr later. During this time they received no further fluid nor food. All subsequent procedures were carried out under well-ventilated fume hoods. One hr after the injection of [^{3}H]water each animal (with the exception of rabbits) was anesthetized with ether. Rabbits were anesthetized by an injection of pentobarbital. The abdomen was immediately opened and an exact aliquot of blood was aspirated from the abdominal aorta. This volume equaled 5 ml in the rats and monkeys, 3 ml in the hamsters, 10 ml in the guinea pigs, and 50 ml in the rabbits. Various organs were then removed, rinsed in cold isotonic saline to remove excess blood, weighed, and then cut into slices 2–3 mm thick with a razor blade. Two or three aliquots of these slices from each organ were then 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, proximal small bowel (proximal half), distal small bowel (distal half), stomach, spleen, lung, colon, whole blood, pancreas, brain (cerebrum), kidney, heart, adipose tissue (retroperitoneal fat), and muscle (medial thigh muscle). After removal of these organs the animal was skinned and this entire tissue (hair, skin, and subcutaneous fat) was weighed and saponified. The entire remaining carcass, along with the washed-out contents of the gastrointestinal tract was saponified. A portion of the whole blood was centrifuged to obtain plasma from which the SA of plasma water was determined. Since no tissue was discarded, "whole body" sterol synthesis rates could be calculated as the sum of contents of newly synthesized sterol in the individual organs (13).

Determination of sterol synthesis rates in vitro

Rates of sterol synthesis also were measured in vitro in the following tissues: liver, adrenal gland, ovary, proximal small bowel, distal small bowel, kidney, spleen, lung, skeletal muscle, and brain. In these studies the animals were killed and the tissues were immediately removed, chilled, and sliced as previously described (19). Duplicate aliquots of slices weighing 300 mg (the entire, sliced adrenal glands and ovaries were used) were placed in glass tubes containing Krebs' bicarbonate buffer, glucose (5.6 mM), and 20 mCi of [^{3}H]water. The tubes were gassed with 95% O_2:5% CO_2, capped, and incubated in metabolic shakers set at 160 oscillations/min and 37°C for 1.5 hr (19, 23).

Determination of the ^{3}H/C incorporation ratio

This series of experiments was performed to determine the ratio of ^{3}H to carbon (C) incorporation into digitonin-precipitable sterols (DPS) during sterol synthesis in the liver. Animals were killed and the livers were immediately removed, chilled, and sliced as above. Three-hundred mg aliquots of slices were incubated in 25-ml center-well flasks containing 5 ml of Krebs' bicarbonate buffer, sodium octanoate (1.0 mM), and either 20 mCi of [^{3}H]water or 4 μCi [1-^{14}C]octanoate (New England Nuclear, Boston, MA). The flasks were gassed with 95% O_2:5% CO_2 and incubated for 1.5 hr at 37°C in metabolic shakers set at 160 oscillations/min (19).

Analytic procedures

As previously described the aliquots of tissue obtained from the in vivo studies (DPS labeled with ^{3}H) or from the in vitro incubations (DPS labeled with ^{14}C or ^{3}H) were saponified with alcoholic KOH. The sterols were then extracted quantitatively and precipitated as the digitonides. These precipitates 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 (12). In the in vitro experiments utilizing [1-^{14}C]octanoate as the labeled precursor, rates of incorporation of [1-^{14}C]octanoate into ketone bodies also were determined as were the rates of synthesis of β-hydroxybutyrate and acetoacetate. From these two sets of measurements the SA of the ketone bodies was calculated (23, 24).

Determination of the distribution of cholesterol in plasma lipoproteins

Pooled plasma from each species was anticoagulated in dry EDTA and equal volumes were adjusted to densities of 1.006, 1.020, 1.055, 1.095, and 1.21 g/ml and

centrifuged simultaneously at 165,000 *g* for 48 hr. The cholesterol concentration in the top 4.0 ml of each centrifuge tube was then measured using the Lieberman-Burchard method (25).

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 (12, 13) 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. This value was determined directly in the rat (12, 13) and was assumed to be correct in the other species.

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:

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

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).

In the in vivo studies 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 also processed with the other tissues of the carcass. This was accomplished by subtracting the product of the residual blood volume and the concentration of [^{3}H]DPS per ml of whole blood from the total radioactivity in the carcass (13). The residual blood volume was calculated by subtracting the volume of blood aspirated from the aorta from the calculated total blood volume of the animal (13). In most cases this correction equaled less than 10% of the total radioactivity found in the carcass [^{3}H]DPS. Similar corrections were made in all tissues of the rat using ^{51}Cr-labeled red blood cells to directly determine residual blood volumes (13). Such corrections were not necessary in the other species because of the low amounts of [^{3}H]DPS in the blood.

In the studies in which rates of sterol synthesis were measured in tissue slices in vitro, the data were expressed as the nmol of [^{3}H]water incorporated into DPS per hr per g of tissue (nmol/hr per g) using the following relationship:

$$\frac{\text{cpm } [^3\text{H}]\text{DPS}}{(1.5 \text{ hr})(\text{g tissue wt})(\text{SA } [^3\text{H}]\text{water})}.$$

In the studies designed to measure the [^{3}H/C incorporation ratio, the rates of incorporation of [^{3}H]water and [1-^{14}C]octanoate into DPS were simultaneously measured in liver slices in vitro. In slices incubated with [^{3}H]water, synthesis rates were calculated as the nmol of [^{3}H]water incorporated into DPS per hr per g of tissue (nmol/hr per g). In slices incubated with [1-^{14}C]octanoate, the corrected rates of synthesis were calculated in terms of the nmol of C_2 units, i.e., acetyl CoA units, incorporated into DPS per hr per g tissue (nmol of C_2 units/hr per g). These values were calculated by means of the following relationship (19):

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

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 conversion of [1-^{14}C]acetyl CoA to sterols. The factor F corrects for intracellular dilution of the SA of the [1-^{14}C]acetyl CoA pool derived from the [1-^{14}C]octanoate. This factor was determined experimentally in liver slices as the ratio of the actual SA of the acetyl CoA pool (determined from the SA of the newly synthesized ketones) to the theoretical SA that the pool should have if undiluted by unlabeled acetyl CoA units (19, 23).

RESULTS

In order to utilize animals that might manifest marked differences in lipoprotein and cholesterol metabolism in the present studies, various species were chosen that were known to vary considerably in the distribution of plasma cholesterol in different lipoprotein fractions and in sterol biosynthetic capacity. An initial experiment was carried out to quantitate the total plasma cholesterol concentration and the lipoprotein distribution of this cholesterol in the five selected species. These animals, it should be emphasized, were all subjected to light cycling (except the monkeys) and were maintained on a defined, low cholesterol intake for at least 2 weeks before being used in experiments. Any animal that failed to eat well and did not gain weight at a rate equal to its cage-mates was excluded from the study. As shown in **Fig. 1,** the mean total plasma cholesterol concentration was nearly identical in the rats (56 mg/dl), rabbits (57 mg/dl), and guinea pigs (54 mg/dl), but was higher in the monkeys (75 mg/dl) and hamsters (124 mg/dl). There were marked differences, however, in the lipoprotein density classes in which this

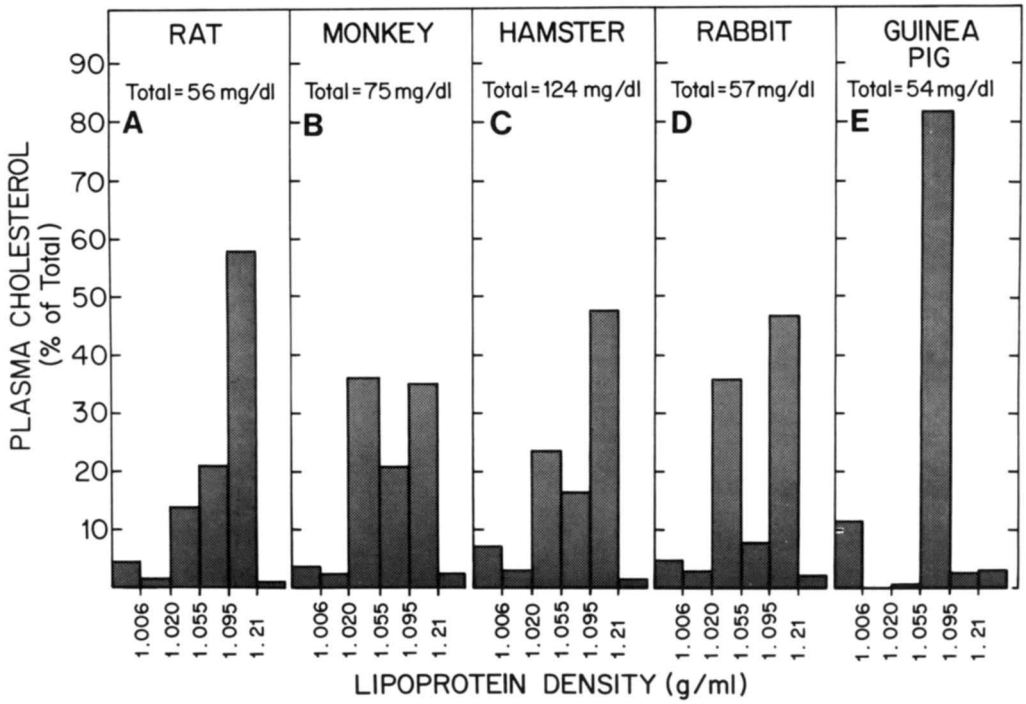

Fig. 1. Distribution of plasma cholesterol among six lipoprotein density fractions in groups of five experimental animals. Pooled plasma was anticoagulated with dry EDTA and equal volumes were adjusted to densities of 1.006, 1.020, 1.055, 1.095, and 1.21 g/ml and centrifuged simultaneously for 48 hr at 165,000 *g*. The cholesterol concentration was then assayed in each density fraction and is expressed here as a percentage of the total plasma cholesterol found in each group of animals.

cholesterol was carried in the different animals. In the rats, for example, most plasma cholesterol was carried in HDL and so floated at a density > 1.095 g/ml (panel A) (26). In contrast, in the guinea pigs there was little HDL and nearly all of the plasma cholesterol was carried in LDL which, in these studies, floated in the ρ = 1.055–1.095 g/ml fraction (27, 28). In the other species, the distribution of cholesterol was more balanced with approximately equal amounts being carried in both the low and high density lipoprotein fractions (panels B, C, and D).

Having chosen these five species in which to quantitate rates of sterol synthesis, it was next necessary to establish that, at least in the liver, the degree of equilibration of the reductive H of NADPH with ^{3}H of the [^{3}H]water in tissue water was essentially the same in these different animals. In order to determine if this critically important condition was, in fact, true, absolute rates of incorporation of [^{3}H]water and [1-^{14}C]acetyl CoA into DPS were determined in vitro in liver slices obtained from rats, hamsters, rabbits, and guinea pigs. Because of the limited number of squirrel monkeys available, this type of experiment was not done in this species. As is evident in column A of **Table 1,** even though all animals were fed up to the time they were killed, there were differences in the rates of ketone body production. Generally livers from the rabbits synthesized ketones at rates that were lower than those seen with the guinea pigs, hamsters, and rats. Despite these differences, however, the SA of the newly synthesized ketones were nearly identical in all groups and varied from 69% to 77% of the theoretical SA that the acetyl CoA pool derived from the [1-^{14}C]octanoate should have if no intracellular dilution had taken place (column B). Thus, the magnitude of the intramitochondrial dilution of the SA of the acetyl CoA pool was of the order of 23% to 31% in the livers of the four species and so was entirely consistent with data previously reported using livers of fed rats incubated with the same 1-mM concentration of [1-^{14}C]octanoate (23, 24, 29). Utilizing these data, the rate of incorporation of [1-^{14}C]octanoate into DPS (column C) found in each liver was corrected for the degree of dilution that took place in that same liver, and these data, in turn, were used to calculate the absolute rates of acetyl CoA incorporation, i.e., C_2 flux, into DPS (column D). When these values were compared to the rates of incorporation of [^{3}H]water into DPS by the same livers (column E), it was possible to calculate the ratio of the number of ^{3}H atoms that were incorporated into the DPS per atom of carbon that entered the biosynthetic pathway as acetyl CoA (column F), or that ultimately was found in the sterol molecule

TABLE 1. Determination of the rates of tritium and carbon incorporation into digitonin precipitable sterols by the liver

Experimental Group	A) Total Ketone Synthesis	B) Relative Ketone Specific Activity	C) [1-^{14}C]-Octanoate Incorporation into DPS	D) Corrected C_2 Flux into DPS	E) [^{3}H]Water Flux into DPS	F) ^{3}H/C Incorporation Ratio	G) ^{3}H/C Ratio in DPS
	μmol/flask		*nmol/flask*	*nmol/flask*	*nmol/flask*		
1) Rat	3.50 ± 0.41	0.74 ± 0.02	39.49 ± 2.57	320.2 ± 26.0	188.2 ± 20.8	0.59 ± 0.02	0.79 ± 0.03
2) Hamster	3.59 ± 0.56	0.77 ± 0.06	10.48 ± 2.49	81.7 ± 14.7	51.7 ± 11.9	0.63 ± 0.04	0.84 ± 0.06
3) Rabbit	2.30 ± 0.41	0.77 ± 0.03	13.14 ± 3.73	102.4 ± 31.3	64.9 ± 17.1	0.63 ± 0.05	0.84 ± 0.07
4) Guinea pig	2.74 ± 0.67	0.69 ± 0.07	5.49 ± 2.15	47.7 ± 15.6	32.4 ± 6.7	0.68 ± 0.07	0.91 ± 0.09

Three-hundred-mg aliquots of liver slices were incubated in 5 ml of Krebs' bicarbonate buffer containing 1 mM sodium octanoate and either 20 mCi of [^{3}H]water or 4 μCi of [1-^{14}C]octanoate. The incubations were carried out in the presence of 95% O_2: 5% CO_2 in a metabolic shaker set at 160 oscillations/min and 37°C, for 1.5 hr. Columns C and E give the rates of incorporation of these two substrates into digitonin precipitable sterols (DPS). Column A shows the rates of ketone body synthesis and column B gives the relative ketone SA expressed as a fraction of the theoretical SA that would have been expected if no intracellular dilution of the acetyl CoA pool had occurred. The corrected C_2 flux into DPS (column D) was calculated from the data in columns B and C as described in the Materials and Methods section. Two types of ^{3}H/C incorporation ratios were then calculated. The ratio of ^{3}H to C incorporated into DPS (column F) was obtained by dividing the values in column E by the values in column D. This ratio gives the μg-atoms of ^{3}H that appeared in the DPS per μg-atom of carbon that entered the biosynthetic pathway as acetyl CoA. The ^{3}H/C ratio in the final cholesterol molecule (column G) was calculated by dividing the data in column F by 0.75 to correct for the 25% loss of C to CO_2 during the conversion of acetyl CoA to cholesterol. This ratio gives the μg-atoms of ^{3}H per μg-atom of carbon found in the sterol molecule. All assays were performed in triplicate and the values represent the mean ± 1 SE for results obtained in liver slices obtained from six to eight animals.

(column G). As is evident, the mean ^{3}H/C incorporation ratios were not significantly different among any of the species and varied from 0.59 to 0.68. These values correspond to the incorporation, on average, of 21 to 24 μg-atoms of ^{3}H from [^{3}H]water into each μmol of cholesterol which, in turn, is consistent with the theoretical value of 22 μg-atoms of ^{3}H that would be incorporated into cholesterol if the reductive H of NADPH did fully equilibrate with the [^{3}H]water (19, 20, 30). Hence, comparison of rates of synthesis of sterol by the liver of these different animal species could be validly made by measuring the rates of incorporation of [^{3}H]water into DPS.

With this information available, the major experiments were next undertaken where rates of sterol synthesis were measured in vivo in the five animal species. Each animal was administered [^{3}H]water intravenously and killed 1 hr later. The amount of newly synthesized [^{3}H]DPS was quantitated in each of the major organs and is shown in **Fig. 2.** In this figure each value represents the nmol of [^{3}H]water incorporated per hr into the DPS present in 1 g of a particular tissue. In the rat, the highest content of [^{3}H]DPS was found in the liver (2279 nmol/hr per g), adrenal gland (1222 nmol/hr per g), and ovary (791 nmol/hr per g). The proximal small bowel (529 nmol/hr per g) and distal small bowel (426 nmol/hr per g) had the next highest contents while the remaining abdominal organs and the lungs, skin, and brain contained lower quantities of newly synthesized sterols. Heart (59 nmol/hr per g), adipose tissue (45 nmol/hr per g), and skeletal muscle (12 nmol/hr per g) contained the lowest amounts of [^{3}H]DPS per g of tissue of any of the organs studied. In these studies the carcass (28 nmol/hr per g) consisted primarily of skeletal muscle, adipose tissue, bone, and marrow.

For comparison, the results obtained in the other four species are shown in panels B–E of Fig. 2. While there was generally a similar pattern of synthetic activity in these various animals there were, nevertheless, some very important differences. The most notable difference was in the content of [^{3}H]DPS in the liver and blood of the various animals. In contrast to rat liver which contained 2279 nmol/hr per g of [^{3}H]DPS, the livers of the monkeys, hamsters, rabbits, and guinea pigs accumulated only 811, 240, 228, and 109 nmol/hr per g, respectively. Similarly, the content of [^{3}H]DPS in whole blood declined from 171 nmol/hr per g in the rats to 83, 29, 26, and 14 nmol/hr per g, respectively, in the monkeys, hamsters, rabbits, and guinea pigs. In contrast to these very large differences found in the liver, the adrenal gland, ovary, and small intestine manifested relatively high contents of newly synthesized sterol in all species; however, there were some interesting quantitative differences. For example, the hamster adrenal gland had the highest content of [^{3}H]DPS found in any organ in any animal species (2495 nmol/hr per g) while the adrenal glands of the rats (1222 nmol/hr per g), guinea pigs (1317 nmol/hr per g), rabbits (853 nmol/hr per g), and monkeys (312 nmol/hr per g) showed lower amounts. The pattern of [^{3}H]DPS content found in the remaining extrahepatic tissues generally was similar among the various species although the tissues of the rat usually had a higher content than the corresponding tissues in the other animals. Finally, it should be noted that there were significant amounts of

newly synthesized sterol found in the brains of the rats, hamsters, rabbits, and guinea pigs (from 66 to 119 nmol/hr per g), all of which were still in an active growth phase during these studies. In contrast, the squirrel monkeys which were young adults with relatively constant weights, manifested essentially no sterol synthesis within their brains (1 nmol/hr per g).

In order to relate these contents, expressed per g of tissue, to the amount of newly synthesized DPS present in each of the major tissues, the values shown in Fig. 2 were multiplied by the individual organ weights which were determined at the time each animal was killed and dissected. This product gave the content of [^{3}H]DPS in each whole tissue. Since all tissues were utilized in these studies, the sum of these "whole tissue contents" gave the total body content of [^{3}H]DPS which, in effect, represented the rate of DPS synthesis in the whole animal. These rates, expressed as the μmol of [^{3}H]water incorporated into DPS per hr by the whole animal, are shown for each species in **Fig. 3.** The rabbits, whose mean weight was 1449 g, had the highest rate of synthesis (100.6 μmol/hr) while the hamsters, whose mean weight was only 167 g, had the lowest rate (8.7 μmol/hr). The content of [^{3}H]DPS in each organ in each animal was then expressed as a percentage of that animal's whole body content and the results of these calculations also are shown in Fig. 3.

Again, the most striking difference among the different species in tissue content of [^{3}H]DPS was seen in the liver which accounted for 51% of the newly synthesized sterol in the rat but much lesser percentages in the monkey (40%), hamster (27%), rabbit (18%), and guinea pig (16%). Of the remaining 15 tissues, 3 were predominantly important in all species as major sites for sterol synthesis: these included the gastrointestinal tract, skin, and carcass. Furthermore, in those species in which the liver made a relatively small contribution to total body sterol synthesis, these 3 tissues became quantitatively much more important as sites for sterol synthesis. The upper gastrointestinal tract (stomach, jejunum, and ileum), for example, contained about 13% of the newly synthesized sterol in the rat but 27% in the guinea pig. Similarly, the carcass accounted for only 13% of the total body content in the rat but 18 to 28% of the [^{3}H]DPS found in the hamster, rabbit, and guinea pig.

In previous work we have shown that the absolute values of the tissue content of [^{3}H]DPS determined in vivo may be influenced by the transfer of [^{3}H]DPS from the sites of synthesis to other organs, even within the relatively short interval of 1 hr over which these measurements were made. In most tissues such transfer was quantitatively minor but in others, such as the adrenal gland, the transfer was very large. In order to examine

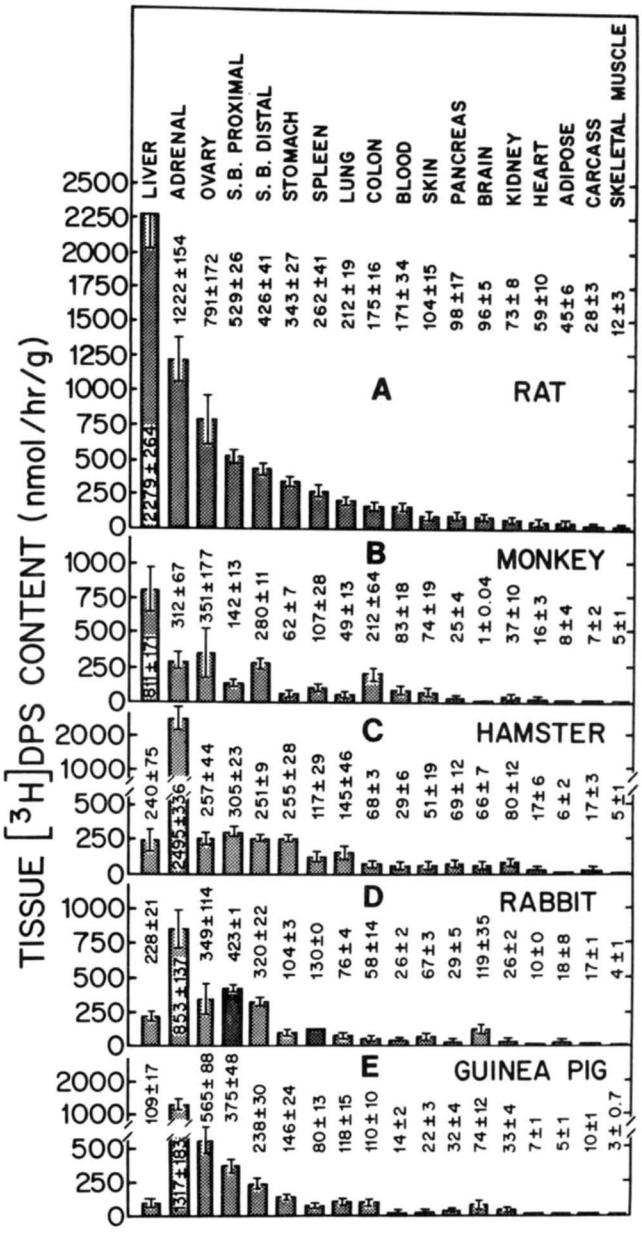

Fig. 2. Content of newly synthesized sterol in the various tissues of the rat, monkey, hamster, rabbit, and guinea pig. All animals were killed 1 hr after the intravenous administration of [^{3}H]water and the tissues were removed, weighed, and assayed for their content of [^{3}H]DPS. These contents are expressed as the nmol of [^{3}H]water incorporated in DPS per hr per g of tissue. The bulk of the adipose tissue and skeletal muscle remained with the residual carcass except for the small aliquots that were used to assay for [^{3}H]DPS content. The data represent mean values ± 1 SEM for results obtained in six rats, three monkeys, four hamsters, three rabbits, and six guinea pigs.

the extent to which such transfers might influence the tissue contents shown in Fig. 2, a final study was undertaken in which rates of sterol synthesis were measured in vitro in slices of ten tissues from four species

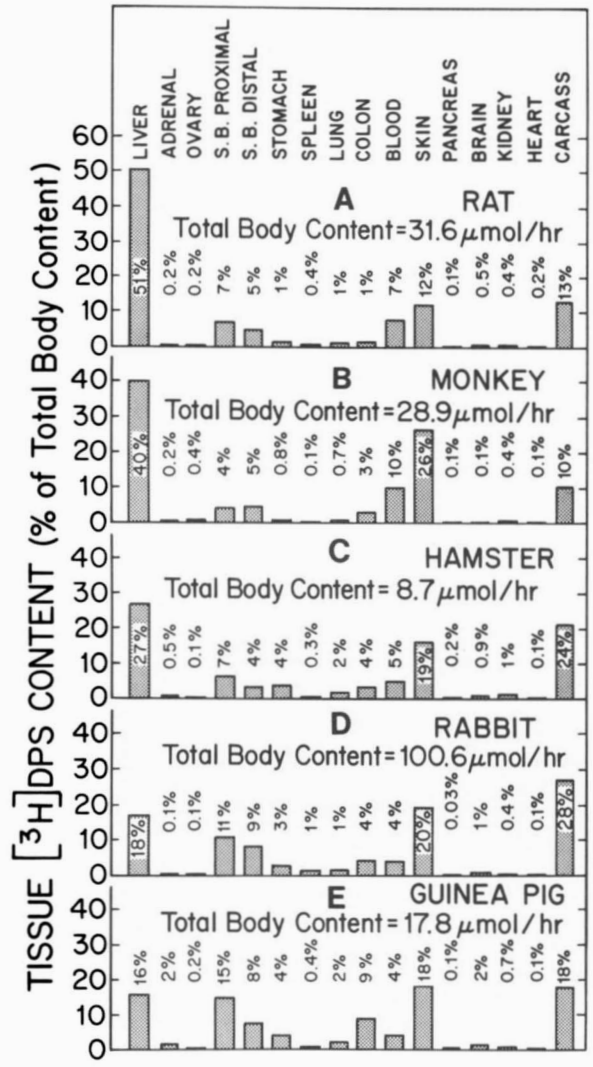

Fig. 3. Content of newly synthesized sterol in the various whole organs of the rat, monkey, hamster, rabbit, and guinea pig. These values equal the product of the tissue [^{3}H]DPS content (nmol/hr per g) shown in Fig. 2 and whole organ weight (g), and are expressed as a percentage of the total body [^{3}H]DPS content. The mean absolute value of the total body [^{3}H]DPS content is also shown for each group of animals and represents the μmol of [^{3}H]water incorporated into DPS per hr. Mean body weights were 200 ± 5 g, 629 ± 10 g, 167 ± 7 g, 1,449 ± 183 g, and 599 ± 13 g for the six rats, three monkeys, four hamsters, three rabbits, and six guinea pigs, respectively.

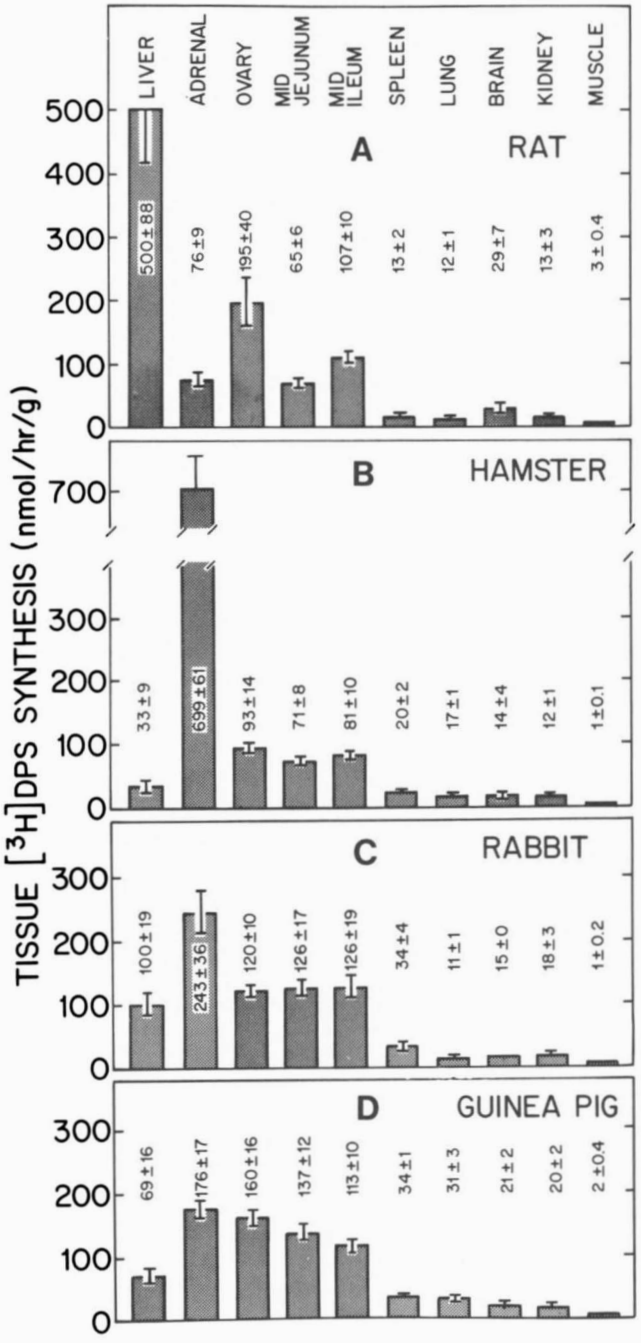

Fig. 4. Determination of rates of sterol synthesis in vitro in ten tissues of the rat, hamster, rabbit, and guinea pig. In these experiments 300-mg aliquots of tissue slices were incubated in 3 ml of Krebs' bicarbonate buffer containing 5.6 mM glucose and 20 mCi of [^{3}H]water. The flasks were gassed with 95% O_2:5% CO_2, capped, and incubated in metabolic shakers set at 160 oscillations/min and 37°C, for 1.5 hr. All values are expressed as the nmol of [^{3}H]water incorporated into DPS per hr per g. These data represent mean values ± 1 SEM for results obtained in six rats, six hamsters, four rabbits, and six guinea pigs.

using [^{3}H]water to circumvent problems of variable substrate uptake and isotope dilution that have marred previous studies of this type. The results of these experiments are shown in **Fig. 4.** Several points deserve emphasis. First, in any species the profile of synthetic activity measured in vitro generally reflected the profile of [^{3}H]DPS content found in vivo (Fig. 2). High rates of synthesis were seen in the liver and small bowel while much lower rates were found in tissues like spleen, lung, brain, kidney, and muscle. In any tissue, however, the absolute rate of synthesis found in vitro was only a fraction of the content of [^{3}H]DPS found in the same tissue in vivo. This difference has been previously noted and studied in detail and reflects the fact that tissues studied

in vitro yield underestimates of the absolute rates of synthesis (12, 19). Second, the in vitro studies also revealed the same marked differences in hepatic sterol synthesis rates as found in the in vivo studies: synthesis in the hamsters, rabbits and guinea pigs occurred at rates that were all less than 20% of the rates found in the rat. Third, there was, however, a major discrepancy between the in vivo and in vitro data with respect to the adrenal gland. In the hamsters, rabbits, and guinea pigs the rates of synthesis in vitro (Fig. 4) generally reflected the contents of [^{3}H]DPS found in vivo (Fig. 2), suggesting that the newly synthesized sterol found in the adrenal gland in vivo came predominantly from local synthesis. In contrast, the high content of [^{3}H]DPS found in vivo in the rat adrenal gland was completely out of proportion to the rate of synthesis found in this organ in vitro, confirming an earlier report that most newly synthesized [^{3}H]DPS found in the adrenal gland in vivo in this species was taken up from the blood and was not synthesized locally (8, 13).

DISCUSSION

These studies were undertaken to quantitate and compare rates of sterol synthesis in the major tissue compartments of five species of animals under in vivo conditions. The validity of these comparisons depends critically upon two fundamental assumptions. The first of these is that the ^{3}H/C incorporation ratio in DPS is the same in all of the tissues of the different experimental animals. This ratio was measured directly in the livers of the rats, hamsters, rabbits, and guinea pigs and was found to vary between 0.59 and 0.68 (Table 1). These values correspond to the situation where approximately 21 to 24 μg-atoms of ^{3}H are incorporated into the cholesterol molecule which, in turn, implies that the reductive H of NADPH is rapidly and essentially completely equilibrated with the ^{3}H of the [^{3}H]water in the cell (19, 20). Similar results have been reported previously in slices of liver (19), in the intact perfused liver (21), and in the liver in vivo (31, 32), where appropriate methods were employed to quantitate absolute rates of acetyl CoA incorporation into sterols. Such results support the conclusion that measurement of rates of incorporation of [^{3}H]water into DPS provide a valid comparison of the rates of hepatic sterol synthesis in the different animal species. Although we have similar data for the intestine, it has not been technically feasible to measure the ^{3}H/C incorporation ratios in every extra hepatic tissue. Thus, it is conceivable that in some of these tissues there is less rapid and complete equilibration of the [^{3}H]water with the reductive H of the pyridine nucleotides. If this is the case, then the error would be in the direction of underestimating rates of sterol synthesis in these peripheral tissues relative to the rates found in the liver.

The second assumption inherent in these studies is that there has been relatively little movement of [^{3}H]DPS from one major tissue compartment to another during the 1-hr interval over which the measurements were made. In a detailed analysis of this problem in the rat, we have previously shown that this assumption is only partially correct (13). For example, when hepatic sterol synthesis was progressively suppressed by the intravenous administration of increasing amounts of cholesterol carried in chylomicrons, the content of [^{3}H]DPS in the blood approached 0 as the content of [^{3}H]DPS in the liver was suppressed toward 0 (13). At the same time, the content of [^{3}H]DPS in many extrahepatic tissues remained unchanged or decreased only slightly while, in a few tissues like the adrenal gland and spleen, the content of [^{3}H]DPS fell in parallel with the content of [^{3}H]DPS in blood. From this type of analysis it was concluded that under the conditions of these experiments, i.e., 1 hr after the intravenous administration of [^{3}H]water, *1*) essentially all of the newly synthesized sterol present in blood had come from the liver, *2*) nearly all the [^{3}H]DPS found in major peripheral tissues such as brain, carcass, and skin had been synthesized in situ, and *3*) much of the [^{3}H]DPS present in a few extrahepatic organs such as the adrenal gland and spleen had been taken up from the blood. At most, however, sterol newly synthesized in the liver accounted for only 19% of the [^{3}H]DPS found in all of the remaining tissues of the body 1 hr after injection of the [^{3}H]water.

From a similar analysis of the data obtained in the other species in the present studies, several major conclusions can be derived concerning these same relationships and the quantitative importance of the major tissues to total body sterol synthesis. The first point concerns the relationship between the content of [^{3}H]DPS in the blood and in the liver in the different groups of animals. As is apparent in Fig. 2, there was a direct relationship between these two parameters: the lower the content of newly synthesized sterol in the liver, the lower the content in the blood. In contrast, there was very little or no correlation between the content of [^{3}H]DPS in the blood and in other major tissues such as intestine, skin, or carcass. This relationship is shown in more graphic terms in **Fig. 5.** The shaded area represents the relationship between the content of [^{3}H]DPS in the blood and in the liver as previously determined in the rat by progressively suppressing hepatic sterol synthesis with cholesterol carried in chylomicrons, as described in the legend to the figure. Thus, as the content of [^{3}H]DPS in the liver of this species was suppressed from approximately 4000 nmol/hr per g to

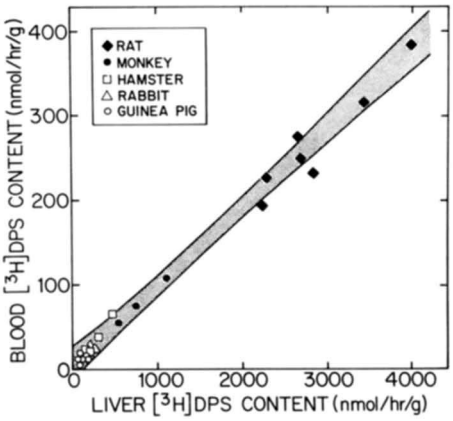

Fig. 5. Relationship between the content of newly synthesized sterols found in the blood and in the liver under in vivo conditions. The blood [^{3}H]DPS content of each animal is plotted against the liver [^{3}H]DPS content of the same animal. The shaded area represents the range of values found previously in the rat where the [^{3}H]DPS content of the liver was varied from approximately 4000 nmol/hr per g down to nearly 0 nmol/hr per g by the intravenous administration of cholesterol carried in chylomicrons. This relationship is given by the formula y = 7.09 + 0.093x and the variation shown by the shaded area equals ± 1 SD (see Fig. 4 of reference 13).

nearly 0 nmol/hr per g, the content in the blood decreased from approximately 400 nmol/hr per g to essentially 0 nmol/hr per g. Superimposed upon this relationship are the data derived from the individual animals used in the present study. It is apparent that all of the animals showed the same relationship, i.e., the content of [^{3}H]DPS in the blood 1 hr after administration of the [^{3}H]water was a direct function of the rate of synthesis in the liver and, as also follows, was essentially independent of the rates of sterol synthesis taking place in nearly all of the extrahepatic tissues. Thus, the content of [^{3}H]DPS in the blood was a direct function of the content in the liver regardless of whether hepatic sterol synthesis was varied in the same species (the rats) or varied spontaneously between different species (the monkeys, hamsters, guinea pigs, and rabbits). This relationship presumably is a consequence of the fact that there is rapid equilibration of the radiolabeled cholesterol across the low resistance, diffusion pathways between the hepatocyte and the lipoproteins in the sinusoidal blood while this equilibrium is much slower across the diffusion barriers in the extrahepatic tissues.

The second major point derived from these studies concerns the relative rates of sterol synthesis that takes place in vivo in the five species of animals and, more particularly, the contribution of synthesis in the liver to such whole animal synthesis rates. The average rate of sterol synthesis in each group of experimental animals equals the total body content of [^{3}H]DPS shown in Fig. 3. These values varied over a wide range, from 8.7 μmol/hr in the hamster to 100.6 μmol/hr in the rabbit, since the mean size of the experimental groups of animals also varied greatly. However, size differences could be factored out by normalizing these data to a constant body weight: the results of such calculations are illustrated in panel A of **Fig. 6** and show the μmol of [^{3}H] water incorporated into [^{3}H]DPS per hr per 100 g of body weight. As is apparent, the rates of whole-animal sterol synthesis in the monkeys, hamsters, rabbits, and guinea pigs were remarkably similar and only varied from 2.9 to 4.6 μmol/hr per 100 g body weight. In contrast, sterol synthesis in the rat occurred at rates that were nearly 4-fold greater (16.1 μmol/hr per 100 g). Assuming that 1.45 C atoms were incorporated into DPS for each ^{3}H atom (Table 1 and References 13 and 19), then these rates would correspond to the synthesis of 12 mg of cholesterol per day per 100 g rat but much lesser amounts (2.2–3.4 mg/day per 100 g body weight) in the four other species. Clearly, the rat is very different in this regard from the monkey, hamster, rabbit, guinea pig, and especially man (who synthesizes about 1 mg of cholesterol/day per 100 g body weight) in its remarkably high capacity for sterol synthesis.

The relative contribution of the liver and extrahepatic tissues to these whole-animal synthesis rates can also be derived from these data and are shown in panels B and C of Fig. 6. Because essentially all of the [^{3}H]DPS in the blood is derived from the liver, the sum of the [^{3}H]DPS contents in these two tissues closely approximates the actual rates of hepatic sterol synthesis in vivo (13). As seen in panel B, the liver of the rat synthesizes sterol at a rate that is from 4 to 16 times higher (per 100 g body weight) than the livers of the other animal species. In contrast, the extrahepatic tissues of the rat contain only about twice the [^{3}H]DPS as do the extrahepatic tissues of the monkeys, hamsters, rabbits, and guinea pigs. Hence, while about half of whole-animal sterol synthesis in the rat takes place in the liver, in all of the other species the liver is quantitatively far less important and is the source of a much smaller percentage of the total [^{3}H]DPS that is synthesized in the whole animal.

Extrapolating from earlier work on relative rates of synthesis that have been measured with ^{14}C-labeled substrates and with [^{3}H]water in human hepatic biopsies, it would appear that in man the liver also makes a relatively small contribution to total body sterol synthesis (17). This conclusion may explain those early studies in man in which it was found that a high dietary cholesterol intake had little or no detectable effect upon total body sterol synthesis, as detected by external balance techniques, even though cholesterol feeding was known to suppress hepatic cholesterol synthesis (16, 33–36). Such a result would be anticipated if the liver made only a small contribution to total body synthesis. Thus, from these various considerations it is apparent that earlier work utilizing various ^{14}C-labeled substrates has seri-

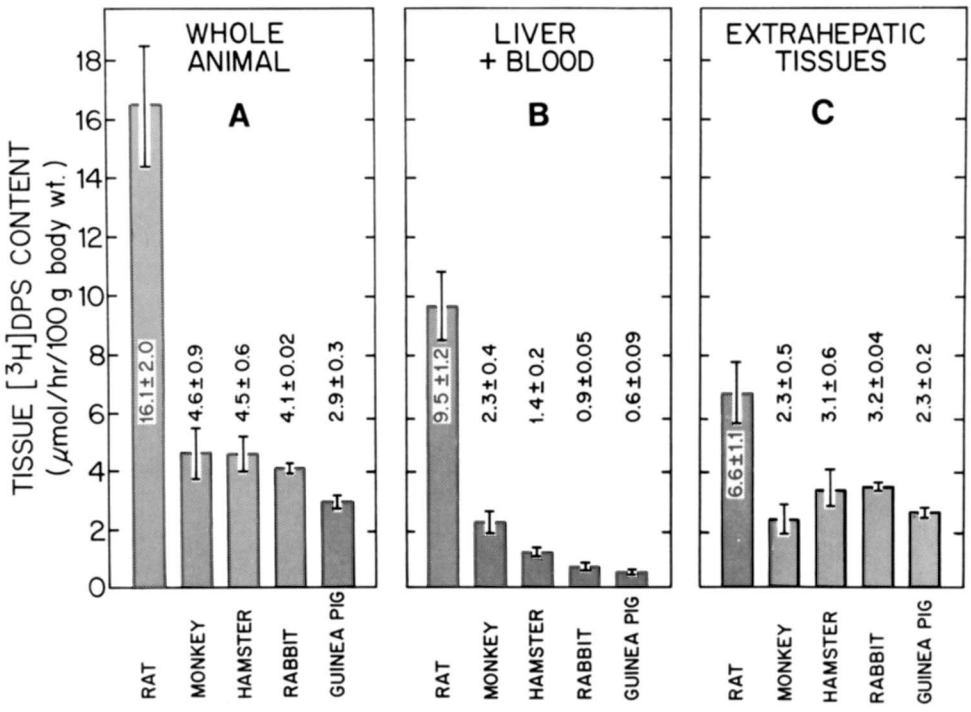

Fig. 6. Content of newly synthesized sterol in the major body compartments normalized to a constant body weight. The data show the amount of [^{3}H]DPS found in the whole animal (panel A) or in the liver and the blood (panel B) and the remaining extrahepatic tissues (panel C) when these values are normalized to a constant animal weight of 100 g. The data represent mean values ± 1 SEM for results obtained in six rats, three monkeys, four hamsters, three rabbits, and six guinea pigs.

ously overestimated the quantitative importance of the liver to whole-animal sterol synthesis.

If this is the case, then the third point to be derived from these studies is that most of the [^{3}H]DPS found in the extrahepatic tissues was synthesized locally and did not come ultimately from the liver. Even in the rat, in which the liver has an exceptionally high rate of synthesis (Fig. 6), only about 19% of the newly synthesized sterol present in all of the extrahepatic tissues was derived from the liver (19). In the other species where the absolute and relative rates of hepatic sterol synthesis are much lower, it follows that the overwhelming majority of the [^{3}H]DPS found in the extrahepatic tissues must have been synthesized in situ in those tissues.

The most dramatic example of this difference in the source of newly synthesized sterol in extrahepatic tissues in the different species is seen in the adrenal gland. In the rat, previous data have shown that about 96% of the [^{3}H]DPS present in this endocrine gland came from the blood (and hence the liver). Thus, as shown by the shaded area in **Fig. 7,** when the content of [^{3}H]DPS in the blood was lowered from about 240 nmol/hr per g to essentially 0 nmol/hr per g (by suppression of hepatic sterol synthesis), the content of [^{3}H]DPS in the adrenal gland also was reduced essentially to 0 nmol/hr per g. The data derived from the adrenal glands of the rats and monkeys used in the present study are nearly superimposable upon these data. In contrast, the adrenal glands of the hamsters, rabbits, and guinea pigs manifested much higher contents of [^{3}H]DPS than would be expected from the low amounts of [^{3}H]DPS present in the blood of these animals. Such results suggest that while the adrenal glands of all five species had very high contents of newly synthesized sterols under in vivo conditions (Fig. 2), this [^{3}H]DPS was derived mainly from the blood (and hence the liver) in the rat and monkey but was largely synthesized locally in the hamsters, rabbits, and guinea pigs. This conclusion was supported by the in vitro incubation studies (Fig. 4) where the rates of [^{3}H]DPS synthesis were many fold higher in the adrenal glands taken from these latter three species than from the rat.

As is shown in Fig. 3, the three principal sites in all five animal species for sterol synthesis in the extrahepatic compartment were the small intestine (jejunum and ileum), skin, and carcass. The content of [^{3}H]DPS in the small intestine clearly underestimates the actual importance of this organ to total body sterol synthesis since a portion of the newly synthesized sterol must have already moved out of the intestine (presumably in chylomicrons) during the 1-h experimental period. Thus, in the rat, the content of [^{3}H]DPS in the small intestine was found to equal about 12% of the total body content; however, since it could be shown that an equal amount

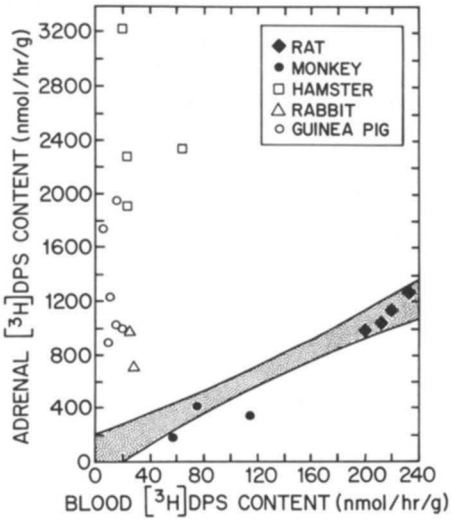

Fig. 7. Relationship between the content of [^{3}H]DPS found in the adrenal gland and in the blood under in vivo conditions. The [^{3}H]DPS content of the adrenal gland is plotted against the [^{3}H]DPS content of the blood in the same animal. The shaded area represents the range of values found previously in the rat where the [^{3}H]DPS content of the blood was varied from approximately 240 nmol/hr per g to nearly 0 nmol/hr per g. This relationship is given by the formula $y = 49.9 + 4.88\times$ and the variation shown by the shaded area equals ± 1 SD (see Fig. 7 of reference 13).

of [^{3}H]DPS had already moved out of the intestine, it was determined that this organ was actually responsible for the synthesis of about 24% of the sterol that was synthesized in the whole animal (13). In contrast to the intestine, there was little movement of newly synthesized sterol out of the carcass compartment (striated muscle, marrow, and bone) so that the content of [^{3}H]DPS in this tissue closely approximated its actual contribution to total body sterol synthesis (13). In the rat this contribution equaled about 13% of the total body synthesis but in the rabbit approached 28%. The physiological significance of sterol synthesis in the skin is more difficult to interpret. Much of the sterol is not cholesterol (37) and furthermore it is likely that a significant percentage is lost directly to the outside of the animal through the sloughing of cells, hair, and oily secretions from the skin. Hence, presumably only a small fraction of the skin [^{3}H]DPS may enter into the miscible pools of body cholesterol.

From these various considerations it is apparent that the major sites for sterol synthesis in animals like the rabbit, guinea pig, and hamster (and, possibly, the baboon and man (17)) are the gastrointestinal tract and the carcass (principally muscle). This conclusion is very different from that reported earlier from several laboratories where ^{14}C-labeled substrates were used to quantitate rates of sterol synthesis in different tissues under in vitro conditions (14, 37, 38). Such studies grossly underestimated the importance of most extrahepatic tissues (muscle, in particular) to total body synthesis.

In summary, these studies point up very important quantitative differences in the sites of sterol synthesis among the various species that were studied and, in particular, between the rat and the other species. The liver of the rat has an exceptionally high rate of sterol synthesis and makes an important contribution to the newly synthesized pool of sterol in the extrahepatic tissues. Hence, in this species it has been found that approximately half of low density lipoprotein uptake and degradation takes place in extrahepatic tissues (11); in such tissues as the adrenal gland most of the cholesterol that is utilized is taken up from lipoproteins, either low or high density lipoproteins (6). In contrast, in a species such as the hamster, the liver makes little contribution to the newly synthesized sterol pool in the extrahepatic tissues. As a correlate of this it has been shown that most low density lipoprotein is taken up and degraded in the liver, and that there is relatively little lipoprotein cholesterol uptake in peripheral tissues such as the adrenal gland. When subjected to excessive loss of cholesterol (through cholestyramine feeding), the liver of the hamster, with its limited capacity to synthesize sterol, responds by increasing the rate of low density lipoprotein uptake whereas in the rat hepatic sterol synthesis increases to meet the new demands and there is no change in the kinetics of low density lipoprotein turnover. Thus, it is apparent from these few examples that the kinetics of lipoprotein turnover, the regulation of lipoprotein degradation, and the rates of sterol synthesis in various tissues are closely linked and therefore the characteristics of each of these processes may vary markedly among the different species of experimental animals.

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