Measurement of rates of cholesterol synthesis using tritiated water

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Abstract Rates of sterol synthesis in various tissues commonly are assessed by assaying levels of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase on isolated microsomes or by measuring the rates of incorporation of various ^{14}C-labeled substrates or [^{3}H]water into cholesterol by whole cell preparations in vitro or by the tissues of the whole animal in vivo. While measurement of activities of HMG-CoA reductase or rates of incorporation of ^{14}C-labeled substrates into cholesterol give useful *relative* rates of sterol production, neither method yields absolute rates of cholesterol synthesis. The use of [^{3}H]water circumvents the problem of variable and unknown dilution of the specific activity of the precursor pool encountered when ^{14}C-labeled substrates are used and does yield *absolute* rates of cholesterol synthesis provided that the ^{3}H/C incorporation ratio is known for a particular tissue. In 12 different experimental situations it has been found that from 21 to 27 μg atoms of ^{3}H are incorporated into cholesterol from [^{3}H]water in different tissues of several animal species, so that the ^{3}H/C incorporation ratio is similar under nearly all experimental conditions and varies from 0.78 to 1.00. When administered in vivo, [^{3}H]water rapidly equilibrates with intracellular water and is incorporated into sterols within the various organs at rates that are linear with respect to time. From such data it is possible to obtain absolute rates of cholesterol synthesis in the whole animal and in the various organs of the animal. Current data suggest, therefore, that use of [^{3}H]water yields the most accurate rates of cholesterol synthesis both in vitro and in vivo.—**Dietschy, J. M., and D. K. Spady.** Measurement of rates of cholesterol synthesis using tritiated water. *J. Lipid Res.* 1984. **25:** 1469–1476.

Supplementary key words liver • intestine • ^{3}H/C incorporation ratios

Studies designed to elucidate the mechanisms of cholesterol balance in individual cells and tissues and in the whole animal require valid methods for accurately quantitating rates of sterol synthesis under both in vitro and in vivo conditions. Generally, three types of methods have been used and these include measurement of the activity of microsomal 3-hydroxy-3-methylglutaryl (HMG) CoA reductase activity (1), rates of incorporation of various ^{14}C-labeled precursors into cholesterol (2), and rates of incorporation of ^{3}H from [^{3}H]water into sterols (3). While each of these methods generally yields valid measurements of *relative* changes in rates of cholesterol synthesis under different experimental conditions, each method may, under certain circumstances, give erroneous data that do not reflect changes in either the *relative* or *absolute* rates of sterol synthesis under a given experimental condition. This review will briefly discuss the advantages and various technical problems associated with the use of [^{3}H]water in measuring rates of cholesterol synthesis, both in vitro and in vivo.

Theoretical considerations

Measuring HMG-CoA reductase activity at what are assumed to be saturating levels of substrate remains a useful and easy technique for assessing relative changes in rates of cholesterol synthesis in a given tissue preparation. Such a technique, however, usually grossly underestimates the absolute rate at which that tissue synthesizes sterol (1). Furthermore, because of differences in the recovery of microsomes and of enzyme kinetics in different tissues, there may be errors in assessing even relative rates of sterol synthesis in different cell preparations and tissues by this method. Finally, measurements of HMG-CoA reductase activity cannot be used to assess the effects of various inhibitors on sterol synthesis in vivo if the effect of such inhibitors is reversible and lost during the preparation of microsomes.

A second, commonly used technique is to measure the rate of incorporation of a ^{14}C-labeled substrate such as acetate, glucose, pyruvate, or octanoate into cholesterol. Again, this technique is simple and still useful, although subject to potential errors under certain circumstances. As illustrated in **Fig. 1,** such ^{14}C-labeled substrates must be taken up into the cell (by either passive or carrier-mediated processes) and then metabolized to [^{14}C]acetyl CoA in either the mitochondrial compartment, e.g., [^{14}C]octanoate, or cytosolic compartment, e.g., [^{14}C]acetate. At any point along these pathways unlabeled intermediate compounds arising

Abbreviations: HMG, 3-hydroxy-3-methylglutaryl; DPS, digitonin-precipitable sterols; SA, specific activity.

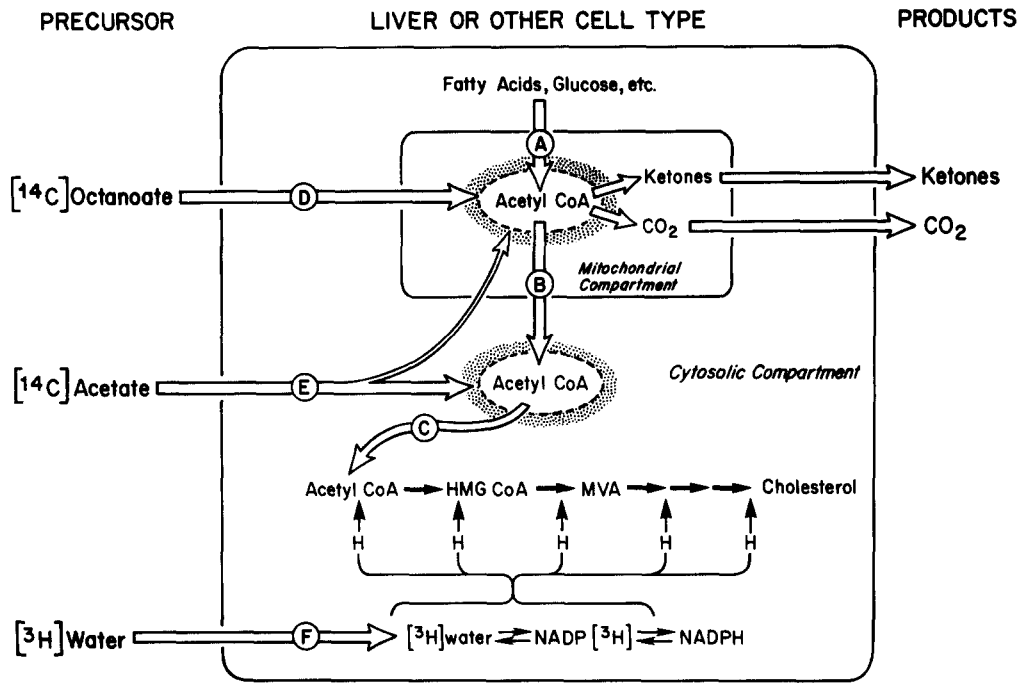

Fig. 1. Pathways for the incorporation of various radiolabeled precursors into cholesterol. In this diagram, the pool of acetyl CoA in the cytosolic compartment that is the substrate for cholesterol biosynthesis is assumed to be distinct from the intramitochondrial pool of acetyl CoA that is derived from the metabolism of various endogenous substrates including fatty acids and sugars. Hydrogen atoms are inserted into the cholesterol molecule at various steps in the biosynthetic pathway. The extent to which the cholesterol molecule will become labeled with ^3H from [^3H]water will be determined, to a large extent, by the degree of equilibration of the reductive H of NADPH with the ^3H of [^3H]water, as discussed in the text.

from the metabolism of unlabeled precursors in the system may mix with the [^{14}C]intermediate compounds and reduce their specific activity (SA). For example, unlabeled acetyl-CoA derived from the oxidation of fatty acids (pathways A and B) may grossly dilute the SA of the cytosolic pool of acetyl CoA derived from [^{14}C]acetate (pathway E) (4). Hence, the rate of cholesterol synthesis in a given tissue will always be underestimated to the extent that the SA of the cytosolic precursor pool of acetyl CoA used for cholesterol biosynthesis is diluted below the SA of the radiolabeled precursor added to the system. The degree of dilution, and hence the apparent rate of cholesterol synthesis, can vary markedly with different substrates. For example, the apparent rate of incorporation of acetyl CoA units into cholesterol by the same liver slices equals 17, 290, and 363 nmol/hr per g from ^{14}C-labeled glucose, acetate, and octanoate, respectively (3). These marked differences do not reflect variation in rates of sterol synthesis, however, but only variation in the degree of dilution of the SA of the cytosolic [^{14}C]acetyl CoA pool derived from these three precursors. To the extent that such dilution takes place in a given tissue and, further, that the magnitude of this dilution varies in different organs or in the same organ under different metabolic conditions, there may be serious errors in assaying both absolute and relative rates of cholesterol synthesis using ^{14}C-labeled substrates.

Under certain circumstances it is possible to correct for this dilution and so obtain absolute rates of cholesterol synthesis. Under physiologic conditions, most of the cytosolic acetyl CoA used for cholesterol synthesis is ultimately derived from the metabolism of various substrates in the mitochondria (Fig. 1, pathway B) (5). This intramitochondrial pool of acetyl CoA can be labeled with precursors such as [^{14}C]octanoate (pathway D) and its SA can be directly assessed by measuring the SA of another product of this pool such as ketones or CO_2. From such data it is then possible to calculate absolute rates of sterol synthesis in tissues like liver and intestine (4, 6, 7). However, such measurements are technically difficult and time-consuming, and are applicable to only a few tissues under in vitro conditions. Certainly no ^{14}C-labeled substrate can be used to measure either absolute or relative rates of cholesterol synthesis under in vivo conditions because of the variability in rates of tissue uptake of these substrates and the marked variability in the degree of dilution of the SA of the intracellular acetyl CoA pools derived from these substrates.

A third alternative, as also shown in Fig. 1, is to take advantage of the fact that during the biosynthesis of cholesterol (and fatty acids (8)), ^3H atoms from [^3H]water are incorporated into stable, non-exchangeable positions on the sterol molecule. Since [^3H]water freely and rapidly penetrates nearly all cell membranes in the body and since relatively little unlabeled water is generated metabolically within the cells of the different organs, the SA of the intracellular pool of [^3H]water incorporated into cholesterol can be assumed to equal the SA of the [^3H]water found in the extracellular pool in the incubation medium (in vitro) or plasma (in vivo). Hence, the major problem of variable and largely unmeasurable dilution of the SA of the intracellular precursor pool encountered with ^{14}C-labeled substrates is circumvented by the use of [^3H]water. Rates of cholesterol synthesis can be calculated directly from the SA of the [^3H]water present in the bulk fluid phase of the system. However, the use of [^3H]water introduces another, new possible source of error. In measuring relative rates of cholesterol synthesis in different organs or in the same tissue under different metabolic conditions, it must be assumed that the number of ^3H atoms incorporated into the sterol molecule is the same in different organs under the different metabolic conditions. Furthermore, if these rates of incorporation of ^3H into cholesterol are to be converted to absolute rates of sterol synthesis, then the absolute value of the $^3H/C$ incorporation ratio must be known.

$^3H/C$ incorporation ratio

It is technically difficult to determine the number of ^3H atoms that become part of the cholesterol molecule since it is necessary to measure the rates of ^3H incorporation under circumstances where either the absolute rate of cholesterol synthesis can also be measured or where all sterol in the system can be assumed to be newly synthesized. As summarized in **Table 1,** a number of different techniques have been employed by different investigators to obtain such values.

As the cholesterol molecule is synthesized, 18 acetyl CoA units containing 36 carbon atoms are utilized to synthesize one molecule of cholesterol containing only 27 carbon atoms and 46 hydrogen atoms. During this biosynthetic process hydrogen atoms from water are incorporated into the sterol molecule in three different ways. Seven atoms of H are incorporated into the cholesterol molecule directly from water while another 15 atoms are inserted from NADPH during various reductive steps in the biosynthetic pathway (9). Hydrogen atoms from water may also become incorporated into substrates that, ultimately, are used for the generation of the cytosolic acetyl CoA pool used for cholesterol biosynthesis. Thus, it is difficult to predict exactly how many ^3H atoms will be incorporated into each cholesterol molecule on a theoretical basis. For example, if no ^3H from [^3H]water were incorporated into precursors of the cytosolic acetyl CoA pool and if the reductive H of

TABLE 1. Reported values for the $^3H/C$ incorporation ratios in different tissues of various animal species

Species	Tissue	μg Atoms of ^3H per μmol of Sterol	$^3H/C$ Incorporation Ratio		Year (Reference)
			Per Carbon Atom in Acetyl CoA	Per Carbon Atom in the Cholesterol Molecule	
A) Theoretical value		7	0.19	0.26	1977 (9)
B) Theoretical value		22	0.61	0.81	1977 (9)
C) Theoretical value		>22	>0.61	>0.81	
D) Mouse*	Whole animal (in vivo)	21	0.58	0.78	1937 (14)
E) Rat*	Whole animal (in vivo)	23	0.64	0.85	1950 (15)
F) Rat	Liver (in vivo)	24	0.67	0.89	1958 (16)
G) Rat	Brain and liver (in vivo)	21	0.58	0.78	1984
H) Rat	Perfused liver (in vitro)	27	0.76	1.00	1972 (18)
I) Rat	Sliced liver (in vitro)	25	0.69	0.93	1979 (3)
J) Hamster	Sliced liver (in vitro)	23	0.63	0.84	1983 (19)
K) Rabbit	Sliced liver (in vitro)	23	0.63	0.84	1983 (19)
L) Guinea pig	Sliced liver (in vitro)	24	0.68	0.91	1983 (19)
M) Rat	Sliced intestine (in vitro)	25	0.69	0.91	1983 (7)
N) Rat	Isolated hepatocytes (in vitro)	23	0.63	0.84	1983 (20)
O) Hamster*	Ovarian cells (in vitro)	22	0.61	0.81	1984 (17)

This table presents published data on the number of ^3H (or deuterium) atoms that are incorporated into the cholesterol molecule during sterol biosynthesis in a variety of organs in different animal species. Several different techniques have been used to obtain these values and are described in the text. The incorporation data are presented in two ways: as the number of ^3H (or deuterium) atoms found in cholesterol per atom of carbon that enters the biosynthetic pathway as acetyl CoA, and as the number of ^3H atoms per atom of carbon found in the newly synthesized cholesterol. The source of each value is referenced. The data identified with an asterisk (lines D, E, and O) were obtained using water enriched with [D]water rather than [^3H]water. The data in line G are unpublished values from this laboratory. The values in line N were calculated from the cited reference. In this latter study, the $^3H/C$ incorporation ratio in freshly isolated hepatocytes equalled 0.56, 0.65, and 0.72 after 2, 3, and 4 hr of incubation, respectively. Thus, the actual $^3H/C$ incorporation ratio must have averaged 0.84 between 2 and 4 hr of incubation.

the NADPH were derived entirely from unlabeled sources, then only 7 μg atoms of ^{3}H would be found in each μmol of cholesterol. In this case, only 0.19 atoms of ^{3}H would be found in the sterol molecule per atom of carbon that entered the biosynthetic pathway as acetyl CoA. Stated differently, there would be only 0.26 μg atoms of ^{3}H per μg atom of carbon found in the newly synthesized sterol molecule. This represents the minimal theoretical ^{3}H/C incorporation ratio that would occur when cholesterol biosynthesis takes place in the presence of [^{3}H]water (Table 1, line A). Alternatively, if the reductive H of NADPH fully equilibrated with [^{3}H]water, then a total of 22 μg atoms of ^{3}H would be incorporated into each μmol of cholesterol giving a final ^{3}H/C incorporation ratio of 0.81 (line B). An even greater value might be obtained if there were significant incorporation of ^{3}H into an important precursor for the acetyl CoA pool (line C).

The greatest source of variability in these theoretical ^{3}H/C incorporation ratios comes from the uncertainty regarding the degree of equilibration of the reductive H of NADPH with the ^{3}H of [^{3}H]water. On the one hand, it has been reported that in liver there is nearly complete equilibration of the reductive H of NADPH with [^{3}H]water (10) so that approximately 22 ^{3}H atoms should be found in each cholesterol molecule. On the other hand, several reports suggest that in different tissues about half of the NADPH is derived from the pentose monophosphate pathway and, therefore, the reductive H should be unlabeled (11–13). In this case, it would be anticipated that only 14 or 15 ^{3}H atoms would be found in each cholesterol molecule. Because of this uncertainty, it is clear that it is impossible to predict the ^{3}H/C incorporation ratio and it is essential, therefore, that such values be obtained experimentally.

This problem has been addressed in several different ways and the results of these studies are summarized in Table 1. The most direct method for determining such values is to determine how many ^{3}H (or deuterium) atoms are incorporated into the cholesterol molecule under circumstances where nearly all of the sterol in the system can be assumed to be newly synthesized as would be true, for example, when the mouse or rat is raised on a cholesterol-free diet and continuously given drinking water containing [^{3}H]water (or deuterated water) at a constant SA. In four such studies performed in vivo, from 21 to 24 ^{3}H (or deuterium) atoms were found in each molecule of sterol isolated from the whole carcass, liver, or brain of these two species (lines D–G) (14–16). Similarly, 22 μg atoms of deuterium were incorporated into each μmol of cholesterol by ovarian cells from the Chinese hamster (CHO-K1 cells) allowed to replicate in vitro in the continuous presence of deuterated water (line O) (17).

A second method involves the quantitation of ^{3}H incorporation rates into sterols under circumstances where the absolute rate of cholesterol synthesis can also be measured. In one such study using the isolated perfused liver, absolute rates of cholesterol synthesis were determined by calculating the SA of the precursor pool of acetyl CoA from the ^{3}H/C incorporation ratios found in the newly synthesized fatty acids and then assuming that the same pool of acetyl CoA gave rise to both cholesterol and fatty acids (18). In other studies using liver slices from several species, the intramitochondrial pool of acetyl CoA was labeled with [^{14}C]octanoate and the absolute SA of this pool was determined by measuring the SA of the ketones derived from this pool (Fig. 1). Since the acetyl CoA pool in the cytosol giving rise to cholesterol is nearly all derived from the intramitochondrial pool (5), this value could be used to calculate absolute rates of cholesterol synthesis in these tissue slices. Using both of these techniques, it has been reported that 23 to 27 ^{3}H atoms are incorporated into each cholesterol molecule under in vitro conditions in the liver and intestine of the rat, hamster, rabbit, and guinea pig (lines H–M) (3, 7, 18, 19).

Finally, in a recent study in isolated hepatocytes, the absolute rate of sterol synthesis was estimated directly from the rate of accumulation of desmosterol in cells exposed to triparanol. In such freshly prepared liver cells, the number of ^{3}H atoms incorporated into cholesterol varied considerably with the time of incubation but can be calculated to have equaled approximately 22 between 2 and 4 hr of incubation when the cells presumably were metabolically the most stable (line N) (20).

Thus, in so far as data are available, there is good agreement using the various techniques in different organs of various species, under both in vivo and in vitro conditions. In most situations 21–25 ^{3}H atoms are incorporated into the cholesterol molecule so that 0.58–0.69 ^{3}H atoms are incorporated into the sterol molecule per C atom entering the biosynthetic pathway as acetyl CoA. This yields a ^{3}H/C incorporation ratio in the newly synthesized cholesterol molecule of 0.78–0.93. Furthermore, in the liver these ratios are constant and independent of the metabolic state of the liver and the rate of sterol synthesis (3) and in both the liver and intestine the number of ^{3}H atoms incorporated into cholesterol is independent of the type and concentration of substrate from which the precursor pool of acetyl CoA is derived (3, 7). Nevertheless, it should be emphasized that ^{3}H/C ratios are not available for all organs and even though in the whole animal, in vivo, 21–24

μg atoms of ^{3}H are incorporated into each μmol of cholesterol that is synthesized (lines D–G), it is still possible that a small tissue that contributes little to total body sterol synthesis may have a ratio that is significantly different from these values.

It should also be noted that there are two values for the ^{3}H/C incorporation ratio in the literature that do not agree with those summarized in Table 1. In one study the isolated liver was perfused with [^{14}C]acetate and the SA of the ketones was assumed to reflect the SA of the precursor of acetyl CoA in the cytosol giving rise to cholesterol. Since the [^{14}C]acetate enters directly into the cytosolic pool (Fig. 1) (4), such a correction underestimates the SA of this precursor and so leads to an overestimation of the absolute rate of cholesterol synthesis and an erroneously low value for the ^{3}H/C incorporation ratio. It is not surprising, therefore, that a ratio of only 0.35 was reported (21). In a second study, a ^{3}H/C incorporation ratio of only 0.54 was found (14.5 ^{3}H atoms were found to have entered the sterol molecule) (9). However, in this study experimental values were obtained only for the incorporation of mevalonate into cholesterol and no direct measurements were made of how many atoms of ^{3}H might have been incorporated prior to HMG-CoA reductase.

Determination of rates of [^{3}H]water incorporation into cholesterol

Since these various data suggest that the ^{3}H/C incorporation ratios are similar in different tissues of various animal species under both in vivo and in vitro conditions, measurement of rates of [^{3}H]water incorporation into cholesterol does represent one of the best methods for measuring both relative and absolute rates of sterol synthesis, particularly under in vivo conditions. There are several potential technical problems, however, associated with such mesurements that should be emphasized.

The first problem encountered is the result of the large amounts of [^{3}H]water that must be used in such experiments. Since there are large amounts of water in most in vitro and in vivo systems, it is usually necessary to use mCi amounts of radiolabeled water in order to obtain sufficiently high SA. This not only necessitates special laboratory precautions in handling and processing samples, but it also raises the possibility that small amounts of [^{3}H]water might be carried through the process used to isolate the product giving erroneously high incorporation rates. When isolating the [^{3}H]cholesterol by processes such as thin-layer or high-performance chromatography, it is essential that the samples go through one or more drying stages, usually in a vacuum oven, to totally eliminate all contaminating [^{3}H]water. When digitonin precipitation is used to isolate sterols, the digitonides must be split with pyridine and the liberated sterols similarly subjected to drying in a vacuum oven (22, 23). Whatever the analytic techniques employed it is essential that appropriate controls be run through the system to insure that there is no contamination.

The second problem encountered involves the determination of the SA of the precursor pool of [^{3}H]water. Conventionally, rates of sterol synthesis are expressed as the nmol or μmol of [^{3}H]water incorporated into cholesterol per unit time per unit weight (23). This calculation, in turn, requires an accurate value for the SA of intracellular water in the system. Since [^{3}H]water equilibrates within minutes across cell membranes, in in vitro systems the SA of the intracellular pool can be taken as equaling the SA of the water in the incubation medium that is sampled during or at the end of the incubation period. The problem is more complex in vivo as illustrated in panel A of **Fig. 2.** Here again there is very rapid equilibration between plasma water and intracellular water after the intravenous administration of [^{3}H]water (22). However, the SA of the plasma water declines during the first 10–20 min after the intravenous administration of the isotope, as plasma water equilibrates more slowly with other pools of bulk water in the body such as bile, intestinal secretions, cerebral spinal fluid, urine, etc. (22). As a consequence, the average SA of the cell water incorporated into cholesterol throughout the period of observation is slightly higher than the SA determined at the end of the experimental period. Thus, in an animal killed 1 hr after the administration of [^{3}H]water, the average SA is 9% higher than the value determined from the plasma water at the termination of the experiment, i.e., the SA of the plasma water determined at 1 hr must be multiplied by a factor of 1.09. At shorter time intervals this correction becomes quantitatively more important while at longer intervals it becomes negligible. However, when the appropriate factor is taken into consideration, then the rate of [^{3}H]water incorporation into sterols in different organs in vivo is a linear function of time as shown in panel B, Fig. 2.

Finally, the third problem of importance concerns the movement of newly synthesized cholesterol between different tissues during in vivo studies. Under in vitro conditions, where the entire tissue and incubation medium can be processed and the sterols isolated, there is little question as to the origin of the [^{3}H]cholesterol. In vivo, however, sterols synthesized in one organ may move through the blood and be taken up by a second tissue even during relatively short time intervals. Such movement may take place by either molecular exchange or net cholesterol (lipoprotein) secretion or uptake.

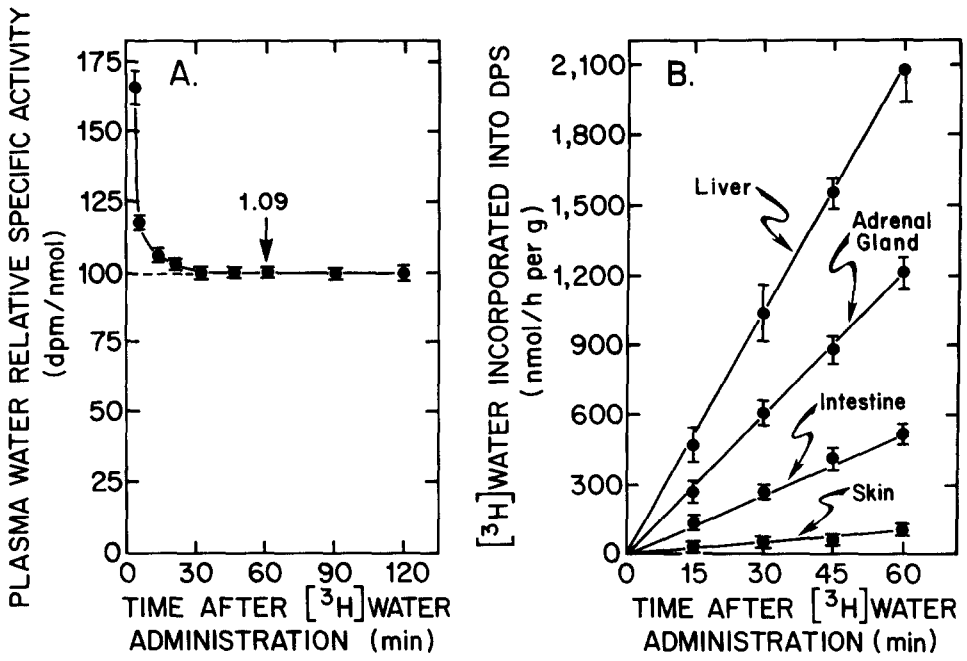

Fig. 2. Plasma water SA and the rates of incorporation of ^3H into digitonin-precipitable sterols (DPS) after the administration of [^3H]water to the rat in vivo. Panel A shows the SA of plasma water at different times after the administration of a single dose of [^3H]water intravenously at time 0. The data have been normalized to a value of 100 dpm/nmol that has been assigned to the value of the constant SA achieved after 30 min. Panel B shows the rates of incorporation of [^3H]water into DPS at different times after the administration of the radiolabeled precursor intravenously at time 0 (22).

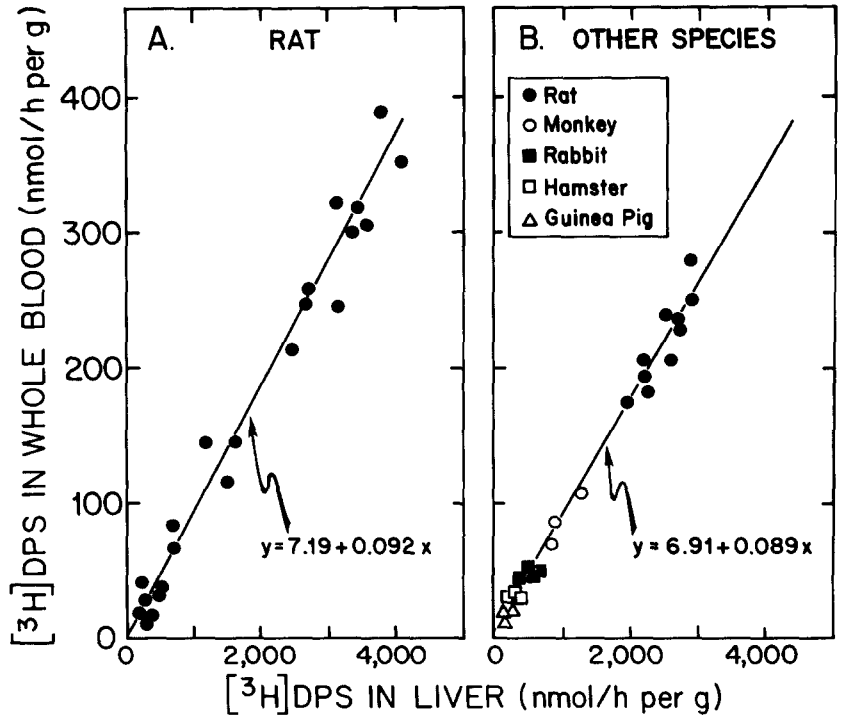

Fig. 3. Content of the ^3H-labeled DPS in whole blood as function of the content of ^3H-labeled DPS in the liver of the rat (panel A) and other species (panel B) 1 hr after the intravenous administration of [^3H]water. In the data shown in panel A the rate of cholesterol synthesis in the liver of the rat was varied over a 500-fold range by feeding either cholesterol or cholestyramine. The data in panel B were obtained in groups of control animals fed a low-cholesterol diet. In this study the variation in the content of ^3H-labeled DPS in the liver of these different species represents the normal variation seen in the intrinsic capacity to synthesize sterols in these different animals (19).

Thus, the amount of newly synthesized cholesterol present in the various organs of an animal after the administration of [^{3}H]water should be expressed as the "content" of [^{3}H]cholesterol in those tissues, and these "contents" are not necessarily a measure of the rates of sterol synthesis in the tissues.

Fortunately, it is also possible to correct for this transfer of newly synthesized sterol between different tissue compartments and so determine actual rates of sterol synthesis in a given organ in vivo. Such corrections are based on the finding that nearly all newly synthesized cholesterol appearing in whole blood 1 hr after the administration of [^{3}H]water comes from the liver. This is illustrated in panel A of **Fig. 3,** where it is evident that newly synthesized sterol essentially disappears from the blood when cholesterol synthesis in the liver is suppressed essentially to zero. This is true even though synthesis in many extrahepatic tissues continues at a high rate (23). A similar relationship is found in other animal species (panel B) where the amount of ^{3}H-labeled DPS in the blood is also a function of the rate of hepatic sterol synthesis in a particular species (19).

A portion of the newly synthesized sterol that moves from the liver into the blood can be taken up by extrahepatic tissues and contribute to their content of [^{3}H]cholesterol. The extent to which this occurs can be experimentally determined by observing the manner in which the content of [^{3}H]cholesterol in a given organ varies with the content of newly synthesized sterol in the blood, as shown in **Fig. 4.** In the rat, for example, an organ like the adrenal gland takes up large amounts of plasma cholesterol so that the content of [^{3}H]-cholesterol in this organ decreases nearly to zero when the content of [^{3}H]sterol in the blood also approaches zero (panel A). Thus, in this endocrine gland, nearly all newly synthesized cholesterol is taken up from the blood and there is essentially no synthesis. In contrast, the content of [^{3}H]cholesterol in tissues like brain is nearly independent of the level of labeled sterols in the blood and so reflects the rate of local synthesis (panel D). Thus, once the proportionality constants are known that describe this relationship in each organ, the absolute rate of sterol synthesis in a given tissue can be calculated by subtracting the product of this constant times the content of [^{3}H]cholesterol in the blood from the content of [^{3}H]cholesterol in that particular organ.

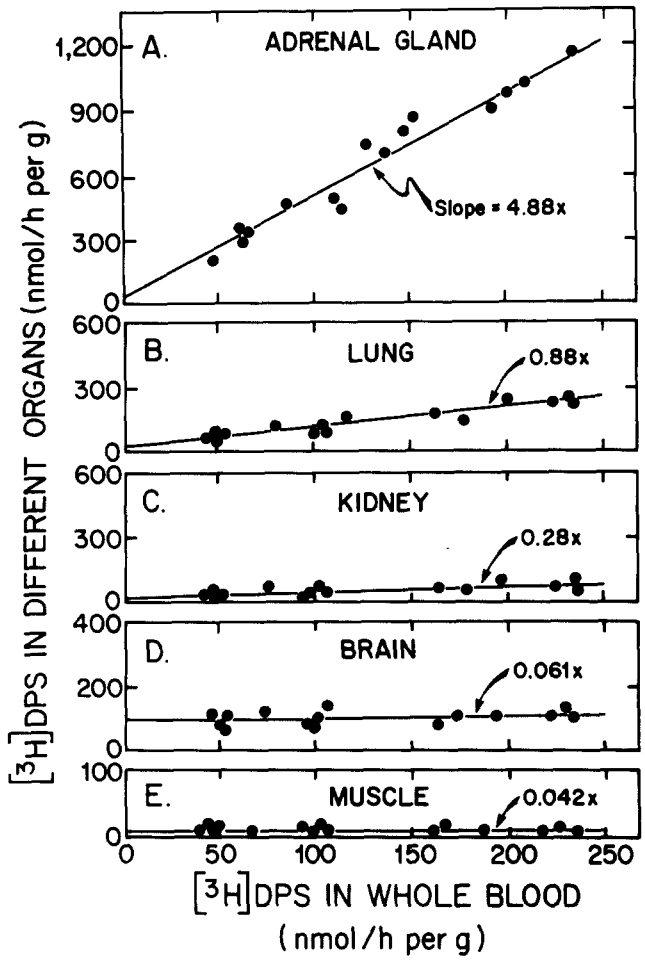

Fig. 4. Content of ^{3}H-labeled DPS in different organs of the rat as a function of the content of ^{3}H-labeled DPS in the circulating blood 1 hr after the intravenous administration of [^{3}H]water. In this study the content of ^{3}H-labeled DPS in whole blood was varied over a wide range by varying the rate of cholesterol synthesis in the liver. The data illustrate the dependency of the content of ^{3}H-labeled DPS in several different organs on the content of ^{3}H-labeled DPS in the circulating blood and, hence, on the rate of sterol synthesis in the liver (23).

Summary

Thus, [^{3}H]water appears to be a very useful substance for measuring rates of cholesterol synthesis in experimental animals, particularly under in vivo conditions. There are a number of technical problems associated with the use of this precursor, however, that must be dealt with and they are described in more detail in recent publications in this journal (3, 19, 22, 23).

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