Sources of cholesterol for kidney and nerve during development

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Abstract Rats were injected intraperitoneally with [^{3}H]water; 2 h later, they were killed, dissected, and cholesterol was isolated from several tissues. Measurement of incorporated radioactivity allowed for calculation of the absolute amount of newly synthesized cholesterol appearing in a tissue. We determined the daily rate of synthesis of cholesterol in the sciatic nerve and kidney of rats at 10 time points between birth and 35 days of age. We compared this to the daily rate of accumulation of total cholesterol. For the sciatic nerve, total accumulation of cholesterol during development was always matched by accumulation of newly synthesized cholesterol, indicating that sciatic nerve synthesizes all of its own cholesterol. This was so independently of whether, at weaning, animals were placed on a cholesterol-free diet or a 2% cholesterol-containing diet. In contrast, in kidney, during the suckling period, only 25% of the accumulated cholesterol was newly synthesized; the remainder came from the circulation. Upon weaning to a 2% cholesterol-containing diet, there was increased local synthesis of cholesterol in kidney, so that within a few days about 50% was locally synthesized. If, however, the animals were weaned onto a cholesterol-free diet, there appeared to be further up-regulation of cholesterol biosynthesis in kidney; now all of the cholesterol accumulating was accounted for by that newly synthesized. Thus, the nerve is invariant with respect to self sufficiency for cholesterol; the kidney changes in this regard during development and as a function of diet. —**Jurevics, H. A., and P. Morell.** Sources of cholesterol for kidney and nerve during development. *J. Lipid Res.* 1994. **35:** 112–120.

Supplementary key words rat • diet • nervous system • [^{3}H]water • cholesterol biosynthesis

Extrahepatic tissues obtain cholesterol needed for assembly of membranes and other functions by a combination of local biosynthesis and uptake of cholesterol-containing lipoproteins from the circulation. Elucidating the mechanism of cholesterol balance in individual tissues thus requires methodology for determination of the rates of sterol biosynthesis in different organs (1). Even under ex vivo conditions, the use of ^{14}C-labeled compounds for determination of absolute rates of synthesis of cholesterol in different organs is problematic; complications arise from differential rates of entry of precursor into cells and partitioning of precursor among various biosynthetic pathways (2). The introduction of the use of [^{3}H]water as precursor made possible determinations of the rate of cholesterol biosynthesis in different tissues in vivo (3–5). With this precursor, the body pool of water rapidly (relative to the time scale of the experiment) reaches equilibrium and remains at constant specific activity.

This approach of using [^{3}H]water, along with manipulations to suppress cholesterol biosynthesis (surgical removal of the small bowel and liver, or intravenous administration of cholesterol-containing chylomicrons), has been used to determine the proportion of newly synthesized cholesterol being supplied locally, relative to that taken in from the blood (4). The ratio of cholesterol provided by local synthesis, relative to that provided by uptake of cholesterol from the blood, varied widely in different tissues.

We have extended the use of [^{3}H]water methods to address the question as to how much of the cholesterol accumulating during development in a particular tissue has been locally synthesized, compared to how much has been provided from the circulation. Studies examining the effects of dietary manipulation of cholesterol intake were also performed.

The experimental design was tested by obtaining data for kidney and sciatic nerve. Kidney was chosen because it has been reported that, in adults, most of the newly synthesized cholesterol comes from the circulation (4). We hoped that this would serve as a control for sciatic nerve, a tissue for which we had indirect evidence suggesting it might be responsible for its own synthesis of cholesterol (see Discussion).

Abbreviation: HPLC, high performance liquid chromatography.

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EXPERIMENTAL PROCEDURES

Materials

[^{3}H]water (100 mCi/ml) was from ICN Radiochemicals, Irvine, CA. All solvents for extraction of lipids and for chromatography were HPLC grade. Isopropanol, acetonitrile, methanol, chloroform, and ScintiVerse E were obtained from Fisher Scientific, Fair Lawn, NY. Cholesterol, ergosterol, squalene, desmosterol, and lanosterol used as standards were from Sigma, St. Louis, MO. Cholesterol-free rat chow and 2% cholesterol rat chow were from ICN Biochemicals, Cleveland, OH.

Animal model

Long-Evans hooded rats were obtained from Charles River Breeding Laboratories (Raleigh, NC) and bred locally. Resultant litters born within 24 h of each other were sexed at 2 days of age and randomly assigned to dams with litter size reduced to 10 male pups for each experimental litter. One litter was maintained to provide filler pups for the experimental litters. All pups were kept with their dams until weaned at age 21 days. Upon weaning, the experimental litters were divided into two groups. One group had ad lib access to a cholesterol-free rat chow, while the other group received a rat chow containing 2% cholesterol. As pups from about 15 days of age consume chow that falls to the bottom of their cage, the dams were given the respective experimental chow from 13 days of age of the pups. All rats were kept on a 12-h dark/light cycle at constant humidity (50 ± 10%) and temperature (21°C ± 2°C). Experimental procedures were performed between 11:00 AM and 1:00 PM during the approximate midpoint of the light cycle, which corresponds to the nadir of hepatic cholesterol synthesis in adult rats (6).

Labeling protocol and preparation of lipid extracts from tissues

At various age intervals from birth to 35 days, one pup from each experimental litter was injected intraperitoneally with [^{3}H]water, 0.3–1.0 mCi/g body weight. After injection, the rats were placed in separate plastic containers without further access to food or water, and kept in a fume-hood for the duration of the labeling period. At the end of the labeling period, rats were killed by decapitation, and 0.5–1.5 ml of blood was collected in 1.5-ml Eppendorf microfuge tubes. Blood samples were centrifuged at 13,000 *g* for 10 min to obtain serum.

Segments of both sciatic nerves, between the lumbar spinal cord and the popliteal fossa, as well as both kidneys were immediately dissected, trimmed of adhering tissue, rinsed of excess blood, and placed in tubes containing 5 ml ice-cold 0.9% saline. Tissues were rinsed 5 more times with the same volume of ice-cold saline, drained on filter paper, and weighed. The kidney, nerve, and serum samples were transferred to clean tubes and total lipids were extracted (7, as modified by 8). A known quantity of ergosterol was added to randomly selected samples to determine the efficiency of the extraction procedure. Recovery was found to be greater then 97%. In some cases lipid extracts were subjected to alkaline hydrolysis. Dried lipid extract corresponding to 10–15 mg wet weight of nerve, 40–50 mg wet weight of kidney, or 50–100 µl serum was suspended in 600 µl, containing equal volumes of water and 10% methanolic potassium hydroxide for 1–2 h at 85°C. Nonsaponifiable lipids were extracted from the mixture with three 2-ml portions of petroleum ether and the combined extracts were dried under nitrogen. The residue was dissolved in chloroform–methanol 1:1. All procedures for dissection of tissue and subsequent extraction of lipids and evaporation of solvent were performed in a fume-hood.

Analytical Methods

Sterols were separated from other lipids by reverse-phase HPLC on a C_{18} column (LiChosorb RP-18, 10 µm, 200 × 4.6 mm; Hewlett-Packard, Kennett Square, PA). The liquid chromatography equipment consisted of a Model IIIG Constrametric pump (LDC Analytical, Riviera Beach, FL), a Model 7125 loading sample injector (Rheodyne, Inc., Cotati, CA) and a one-pump gradient controller (Autochrom, Inc., Milford, MA). The absorbance detector was from ChiraTech Scientific Instruments, Fort Collins, CO. Sterols were eluted isocratically in 45 min at a flow rate of 2 ml/min with acetonitrile–isopropanol 97.5:2.5 and detected by absorbance at 210 nm. After sample elution, the column was washed for 35 min with acetonitrile–isopropanol 40:60 prior to loading a new sample. The UV detector was connected to an on-line integrator (LIPS, Spectrofuge Corp. of North Carolina, Durham, NC) for computer analysis of peak areas. Quantitation of the mass of free cholesterol was made by comparing areas under the cholesterol peaks to areas generated by known quantities of cholesterol. Eluate fractions of 100 sec duration were collected with a 2212-010 Heli Rac (LKB Produkter: Bromma, Sweden). Fractions were dried, 5 ml ScintiVerse E was added, and the radioactivity was determined in a Beckman LS-230 liquid scintillation counter. Counting efficiency for tritium was 26%.

RESULTS

Experimental design

The question asked was, for a given organ in a young rat, how much of the accumulated cholesterol was locally synthesized, and how much came from the blood. The experiment was designed to determine the rate of net accumulation of cholesterol in a particular organ during development, and also to obtain the rate of local synthesis of cholesterol during the same period of time. Accumu-

lated cholesterol not accounted for by local biosynthesis was assumed to be derived from blood.

The rate of accumulation of cholesterol per day in individual organs was calculated from the mass of cholesterol in each organ at various ages. Time units (T) are days and the rate of accumulation between any ages T_1 and T_2 was calculated as:

$$\frac{\text{total cholesterol accumulated}}{\text{day}} = \frac{\text{total cholesterol at } T_2 - \text{total cholesterol at } T_1}{T_2 - T_1} \qquad Eq.\ 1)$$

The rate of accumulation of newly synthesized cholesterol was determined on the basis of label from [^{3}H]water incorporated into cholesterol. The specific activity of water was obtained from the radioactivity in serum, after correcting for its water content which was determined to be 95%. Specific activity (S.A.) of water can be calculated.

$$\text{S.A. of water in dpm/nmole} = \frac{\text{dpm}/\mu\text{l serum}}{(55.5 \text{ nmol water}/\mu\text{l water})(0.95\ \mu\text{l water}/\mu\text{l serum})} \qquad Eq.\ 2)$$

In further calculations, what is required is the mean specific activity of body water over the time interval of labeling. In an independent series of experiments, we found that specific radioactivity of water in serum leveled off within 5-10 min, as expected (4), and thus the measurement at time of killing was used directly.

As body water is present at constant specific activity during the experiment, the number of ^{3}H atoms entering each cholesterol molecule can be calculated and label incorporated into cholesterol can be expressed as molecules of cholesterol synthesized. This calculation requires an assumption as to how many hydrogen atoms in each molecule of cholesterol are derived from [^{3}H]water. As reviewed in detail (1), this number is best taken to be 22, the equivalent of incorporation of hydrogen from 11 nmol of [^{3}H]water per nmol cholesterol (see also Discussion). Therefore, the specific radioactivity of cholesterol synthesized over the time period of the experiment is 11 times that of body water.

$$\text{nmoles of [}^3\text{H]cholesterol} = \frac{\text{dpm in cholesterol}}{\text{specific activity of water} \times 11} \qquad Eq.\ 3)$$

This can be on a whole organ basis or expressed as specific radioactivity, (nmoles [^{3}H]cholesterol/nmoles total cholesterol). In subsequent figures, we express specific radioactivity as the percent of total cholesterol that is radioactive cholesterol, (nmoles [^{3}H]cholesterol/nmole cholesterol) × 100.

One further correction makes it possible to determine how much of the cholesterol deposited in a tissue during the labeling period is newly synthesized. Although all the radioactive cholesterol is newly synthesized, there is also some synthesis after the injection period from unlabeled pools of precursors existing prior to their metabolic equilibration with label from injected [^{3}H]water. This correction is established from the time course studies (see below) as the time lag before incorporation of label becomes linear with time.

$$\frac{\text{time period of labeling}}{\text{time period of labeling} - \text{time lag}} = L \qquad Eq.\ 4)$$

For convenience in calculation, the correction factor, L, for each tissue is given as a multiplier specific for the 2-h incorporation period which was standard. Additionally, the assumption was made that loss of labeled cholesterol from these organs would be negligible during the time course of the experiment. With these assumptions:

$$\text{newly synthesized cholesterol} = [^3\text{H}]\text{cholesterol} \times L \qquad Eq.\ 5)$$

As before, this can be expressed on a sample or whole organ basis, or as a specific radioactivity by dividing by nmoles cholesterol in the sample. Again, it is convenient to express this value as a percentage of total cholesterol that has been newly synthesized during the time course of the experiment, here 2 h: (nmoles newly synthesized cholesterol/nmoles total cholesterol) × 100.

The accumulation of newly synthesized cholesterol per 2-h period can be converted to accumulation per day, and then compared with the accumulation of total cholesterol (given in equation 1).

$$\frac{\text{nmoles newly synthesized cholesterol}}{\text{day}} = \frac{12\ (\text{dpm in cholesterol/2 h}) \times L}{11\ (\text{specific activity of water})} \qquad Eq.\ 6)$$

In order for the newly synthesized cholesterol to be considered equivalent to cholesterol synthesized locally in a specific organ, it was necessary to demonstrate that the radioactive cholesterol was not synthesized elsewhere and then delivered via the circulation. As data in following sections illustrate, the experimental design takes advantage of conditions where the contribution of radioactive cholesterol from the circulation to cholesterol in organs is very low. Such a state pertains when dietary cholesterol levels are high, during suckling (9, 10) or post-weaning, by inclusion of 2% cholesterol in the diet. Post-weaning dietary manipulation also makes possible control experiments involving a cholesterol-free diet.

Weight and cholesterol content of nerve and kidney during development

The weight of kidney and nerve increases during development (**Fig. 1**). The increased rate of growth of these organs at weaning is as expected, because the milk supply is no longer the limiting variable. Cholesterol content of whole kidney and of the anatomically defined region of sciatic nerve (**Fig. 2**), as well as concentration of cholesterol in serum (**Fig. 3**), were determined. Although cholesterol concentration in kidney and nerve is not plotted, this can be calculated from these data and the organ weights in Fig. 1. The much greater concentration of cholesterol in nerve, relative to kidney, is due to the high cholesterol content of myelin; this membranous structure accounts for about half of the dry weight of sciatic nerve (see ref. 11 for review). The cholesterol concentration of serum follows a somewhat complicated pattern but is equivalent to that previously observed by others (10). Notable is the marked decrease in serum cholesterol content as intake of milk is decreased. Neither body weight, organ weight, nor cholesterol content of the various tissues was significantly influenced by whether or not the post-weaning diet was cholesterol-free or contained 2% cholesterol.

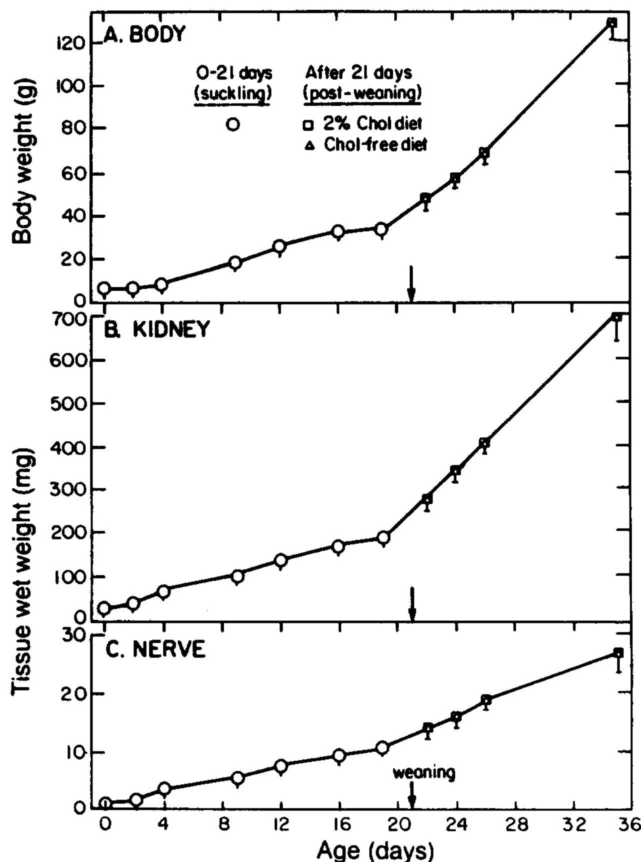

Fig. 1. Weight of body, kidney, and nerve during development. Straight line error bars indicate the range for duplicate values; for times after weaning, bars terminated with a horizontal hatch represent a standard deviation for quadruplicate values. The quadruplicate values result from pooling of the results from animals fed a 2% cholesterol diet and a cholesterol-free diet since these data points always overlapped.

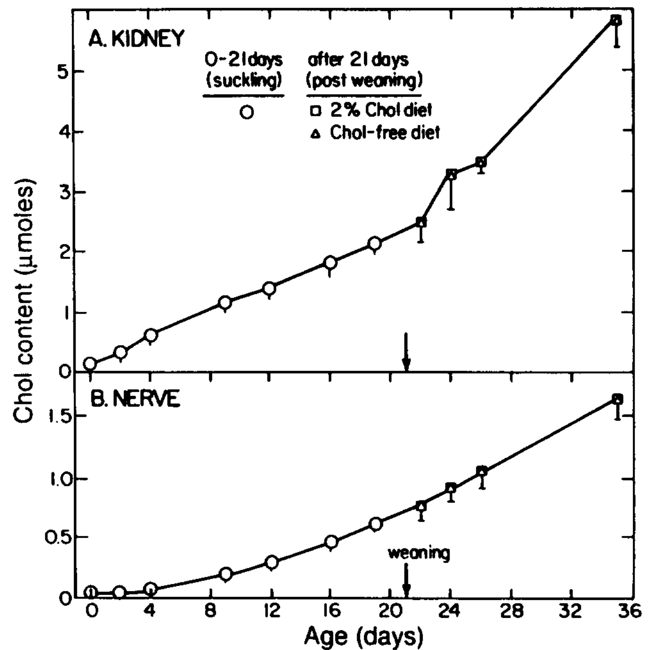

Fig. 2. Cholesterol content of kidney and nerve during development. The error bars are as in the legend for Fig. 1.

Time course of increase in specific radioactivity of cholesterol in different tissues

Representative profiles of optical density and radioactivity from HPLC analysis of sciatic nerve are shown in **Fig. 4.** Cholesterol can be clearly separated from other lipids and quantitated. Some major lipid-soluble precursors of cholesterol are also identified. In a further series

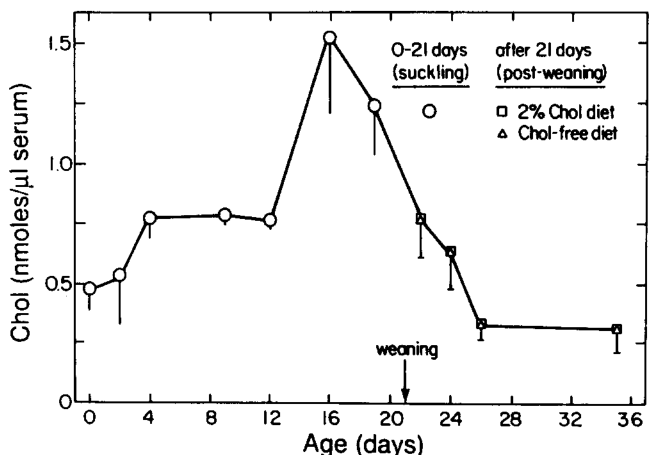

Fig. 3. Cholesterol content of serum. Error bars are as in the legend for Fig. 1.

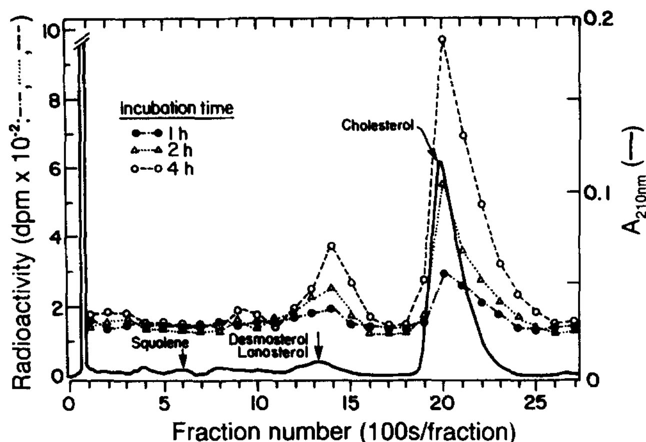

Fig. 4. Elution profile and distribution of radioactivity for cholesterol assay. Lipid extracts from sciatic nerve samples were chromatographed as described in Methods. The absorbance profile of the 1-h sample is shown (the others are identical) as is the radioactivity distribution in samples taken 1 h, 2 h, or 4 h after intraperitoneal injection of 20 mCi [^{3}H]water in 22-day-old rats recently weaned onto a cholesterol-free diet. Each assay involved isolation of lipid from the two sciatic nerves of an animal, and chromatography of one-half of this sample.

of experiments, the time course of incorporation of label into cholesterol of nerve, kidney, and serum was established, at an age when dietary cholesterol could be controlled (**Fig. 5**). Data are plotted as the percent of total cholesterol that was radioactive at various times after injection of [^{3}H]water. This is a convenient way of specifying specific radioactivity while normalizing for variations in injection of [^{3}H]water, and simplifies the relationship to plots in subsequent figures.

The data for serum in the cholesterol-fed animals (Fig. 5A) are as expected; there is little radioactivity in serum cholesterol as synthesis of cholesterol by liver is repressed by dietary cholesterol. When animals are placed on a cholesterol-free diet, specific activity of serum cholesterol increases rapidly. The lack of initial linearity represents the delay in equilibration of label with cellular pools of water and with metabolic intermediates. Note that the results for serum from animals fed a cholesterol-free diet indicate a lag of approximately 40 min before incorporation becomes linear with time. This can be used to obtain a correction factor to allow calculation of the actual rate of synthesis of cholesterol. As subsequent figures present data only from 2-h labeling periods, the correction factor is given as a multiplier (the factor L of equation 4); to account for the 37-min time lag out of 120 min, L is set at 1.4. We note that in this, as in other experiments described herein, the term cholesterol refers to unesterified cholesterol. A series of control experiments were carried out involving alkaline hydrolysis of serum from suckling (16-day-old) rats as well as 22-day-old rats on diets either cholesterol-free or containing 2% cholesterol. Samples at 2 and 6 h post injection were analyzed. In all cases the specific activity of cholesterol in the hydrolyzed sample was from 0 to 15% less than that of the serum unesterified cholesterol.

The data for sciatic nerve (Fig. 5C) is qualitatively different, in that dietary cholesterol did not alter the extent or the time course of incorporation of label into cholesterol. Thus, in either dietary situation, cholesterol accumulating in sciatic nerve is produced only locally as

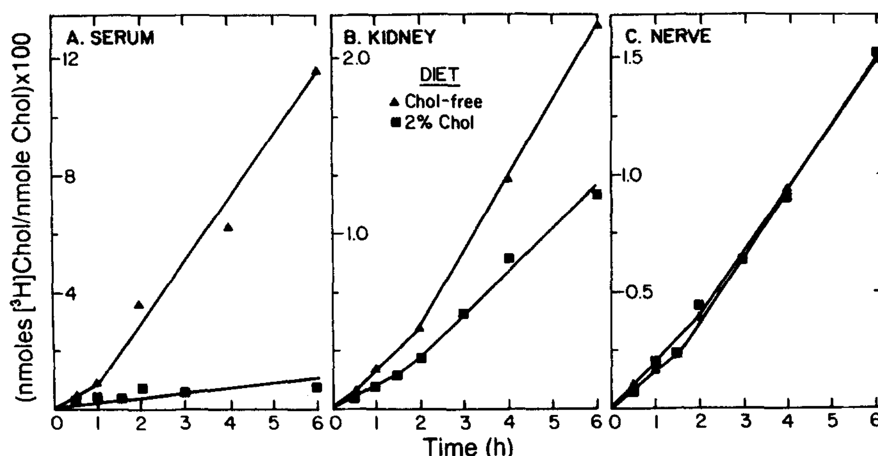

Fig. 5. Percentage of total cholesterol that is labeled as a function of time after injection of [^{3}H]water. Rats were weaned onto either a cholesterol-free diet or one containing 2% cholesterol. At 22 days of age they were injected with 20 mCi of [^{3}H]water. At the times indicated animals were killed, organs were dissected, and total lipids were extracted from serum, kidney, and nerve. Total cholesterol and radioactivity in cholesterol of each sample were determined. As detailed in the Experimental Design section, with knowledge of the specific radioactivity of body water, radioactivity incorporated into cholesterol can be expressed as an equivalent amount of cholesterol that is metabolically labeled. As detailed in equation 3 and following section in the text, the amount of [^{3}H]cholesterol relative to preexisting total cholesterol is a measure of specific radioactivity. This is presented above as a percent of total cholesterol that is newly synthesized.

it is not affected by changes in the specific activity of circulating cholesterol. As above, the points between 2 h and 6 h can also be used to obtain an apparent time lag for equilibration of label in injected water with the pool of metabolic intermediates leading to cholesterol. In subsequent figures, newly synthesized cholesterol is obtained by setting the time lag factor, L (equation 4) at 1.5. We acknowledge that, ideally, this time course correction should be conducted for each organ at age points studied, but preliminary experiments suggest that the variability in this parameter is not great.

The data for kidney (Fig. 5B) are intermediate relative to serum and nerve. Incorporation of label into cholesterol is stimulated in rats on a cholesterol-free diet, relative to animals given 2% cholesterol. The increased radioactivity present in kidney cholesterol of animals on a cholesterol-free diet could be assumed to come, in part, either from increased local synthesis or from uptake of circulating labeled cholesterol. These time courses were used to obtain values for L of 1.6 and 1.9 for the high cholesterol diet or cholesterol-free diet, respectively. We assumed that in suckling rats the metabolic situation for cholesterol biosynthesis in extrahepatic tissues would be similar to that in the 2% cholesterol-fed animals and the same correction factor was used. For both kidney and nerve, the content of esterified cholesterol was trivially low and alkaline hydrolysis did not change the specific activity of the cholesterol as assayed by HPLC.

Newly synthesized cholesterol in different tissues during development and as a function of cholesterol in the diet

The percentage of cholesterol in serum, nerve, and kidney that was newly synthesized was evaluated 2 h after injection of [^{3}H]water at various ages during development. In the serum (**Fig. 6A**), specific radioactivity of cholesterol is relatively low during most of the suckling period and during post-weaning feeding of a high cholesterol diet. The elimination of cholesterol from the diet at weaning causes a rapid and prolonged rise in specific radioactivity of serum cholesterol. This reflects up-regulation of cholesterol synthesis by liver to make up for the dietary deficit. The relatively high specific radioactivity of serum cholesterol during the initial postnatal period is presumably because cholesterol synthesis in fetal liver is high prenatally and is down-regulated postnatally (10). In the case of sciatic nerve (Fig. 6C), the dietary manipulation does not have a significant effect at any post-weaning age point, reinforcing the interpretation of the time course data suggesting that sciatic nerve does not utilize circulating cholesterol. With kidney (Fig. 6B), the dietary manipulation brings about an intermediate effect on specific radioactivity of cholesterol.

In nerve, both pre- and post-weaning and irrespective of cholesterol content of the diet, accumulation of total cholesterol is accounted for by locally produced newly synthesized cholesterol

The rate of accumulation of both total cholesterol (equation 1) and newly synthesized cholesterol (equation 6) were plotted for sciatic nerve and kidney (**Fig. 7**). With respect to the sciatic nerve data, during most of the period of suckling (from a week to 3 weeks postnatally) the total accumulation of cholesterol is largely accounted for by newly synthesized cholesterol (85% at 2 weeks). Could the newly synthesized cholesterol have been synthesized in the liver and extracted from the circulation by sciatic nerve? During most of the suckling period the specific radioactivity of the serum cholesterol pool at 2 h is insignificant relative to the specific activity of cholesterol accumulating in sciatic nerve. This can be ascertained by

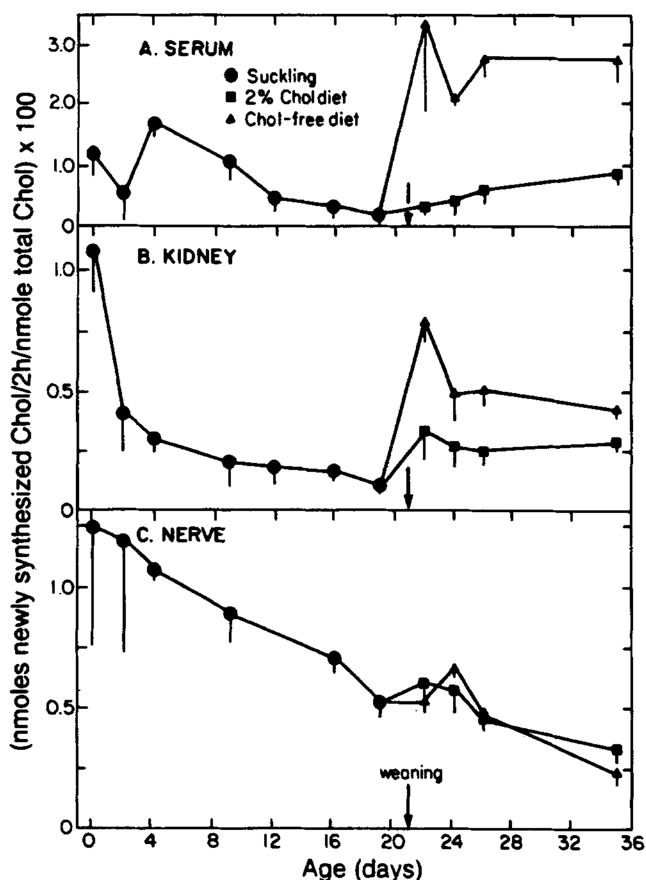

Fig. 6. Percentage of total cholesterol that is newly synthesized in 2 h during development and as a function of diet. Animals of the stated ages were injected with between 0.3 and 1.0 mCi [^{3}H]water/g body weight and 2 h later tissue samples were obtained and specific radioactivity of cholesterol was determined as noted in the text and in the legend to Fig. 5. The bars represent the range for the duplicate determinations.

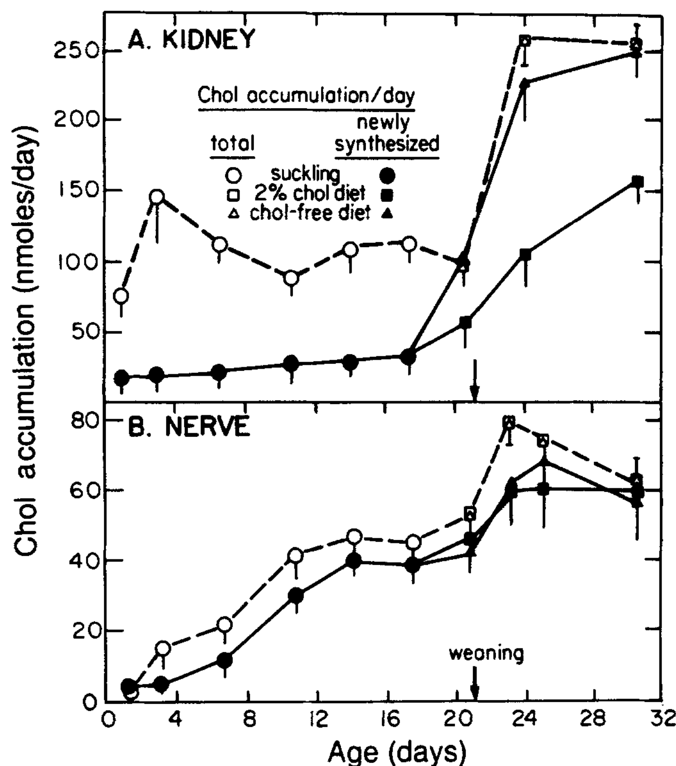

Fig. 7. Total cholesterol accumulated per day and newly synthesized cholesterol accumulated per day during development and as a function of diet. The points representing accumulation of cholesterol per day were obtained from the data set used to create Fig. 2, recalculated to present cholesterol accumulation per day. Error bars for these points are as in the legend to Fig. 1. The lines representing [^{3}H]cholesterol were obtained from the same data base as that used to create Figs. 6B and 6C. This, and other data needed to perform the calculation illustrated in the Experimental Design section allowed for plotting of cholesterol that is newly synthesized in terms of nmoles/day, therefore in the same units as accumulation of total cholesterol. Bars for these data points are the range of duplicate determinations.

noting from Fig. 6A that, during weaning, the specific radioactivity of serum cholesterol corresponds to only 0.5–3.0% being newly synthesized. In contrast, (Fig. 7B) it is apparent that in nerve most of the newly accumulating cholesterol at various developmental stages is newly synthesized at a high specific radioactivity. Thus, the label accumulating must represent cholesterol synthesized in the nerve.

In kidney, most of the cholesterol accumulated prior to weaning originates from the circulation

The above data for nerve are in contrast to the results obtained for kidney. It is apparent from Fig. 7A that during the suckling period most of the accumulated cholesterol is not accounted for by that newly synthesized but instead about three quarters comes from a preexisting source. The source for this cholesterol of low specific activity is presumably in the circulation.

Synthesis of cholesterol by kidney changes after weaning and can be further manipulated by diet, in contrast to the situation in nerve

Our primary experimental design takes advantage of the high cholesterol content of milk during the preweaning period, and of a cholesterol-enriched diet postweaning, which keeps liver suppressed with respect to synthesis of cholesterol. As detailed above, under such conditions newly synthesized cholesterol in an organ could be equated with local synthesis. However, even in the face of continued low specific radioactivity of circulating cholesterol, after weaning the specific radioactivity of cholesterol accumulating in kidney increased considerably from about 25% to over 50% (this can be seen from Fig. 7A upon comparison of the diet-induced change in the ratio of nmoles newly synthesized cholesterol to nmoles accumulated cholesterol).

When specific activity of circulating cholesterol is increased by use of a cholesterol-free diet (Fig. 7A), the total amount of radioactivity in kidney cholesterol is further increased; now all of the accumulated cholesterol is newly synthesized cholesterol. Can this increase in radioactivity be accounted for by uptake of labeled cholesterol from the circulation? Serum cholesterol does indeed increase greatly in specific radioactivity when rats are weaned to a cholesterol-free diet (Fig. 6A). Even the 4- or 6-fold increase, however, raises serum cholesterol specific activity only to a level where 3% of the circulating cholesterol is newly synthesized. This is clearly incompatible with circulating cholesterol being a major source of radioactivity in kidney of animals on cholesterol-free diets, where over 90% of the newly accumulated cholesterol represents that newly synthesized. This leads to the conclusion that the change in diet from high cholesterol during suckling to the cholesterol-free diet up-regulates synthesis in kidney as well as in liver. The signal is not a drastic change in levels of serum unesterified cholesterol which does not seem to alter with diet (Fig. 3). Although we did not routinely quantitate esterified cholesterol, we note it has been shown that such a dietary manipulation does not change the ratio of free to esterified cholesterol in serum (12).

In the sciatic nerve, the post-weaning dietary status makes little difference (Fig. 7B). A cholesterol-free diet did not increase accumulation of label in cholesterol. This is as expected if, regardless of diet, only a small fraction of the cholesterol were coming from the circulation.

DISCUSSION

During development, the size of organs and their net content of cholesterol increase rapidly. We have tested an experimental design that allows us to determine for any given organ whether the needed cholesterol originates

from local synthesis by that organ, or whether it is obtained from cholesterol in the circulation. Our experimental design owes much to a previous investigation (4) that utilized the concept that, after injection of [^{3}H]water into rats, it is possible to determine the absolute amount of newly synthesized cholesterol present in an organ. That study, carried out in adult rats, related the specific activity of circulating cholesterol (manipulated by surgical removal of small intestine and most of the liver or by infusion of chylomicrons) to that in different organs. A strong correlation was taken to imply organ utilization of circulating cholesterol, while a lack of correlation implied organ independence of circulating cholesterol.

Another relevant experimental paradigm involves rearing rat pups on an artificial milk containing deuterium-labeled cholesterol (13). Deuterated cholesterol present in blood and organs could then be evaluated by gas chromatography-mass spectrometry. In that study it was demonstrated that brain does not derive significant cholesterol from the serum during the suckling period (preliminary data from analyses of brain samples of the animals used in our study support this conclusion). This is relevant as in both nerve and brain much of the accumulation of cholesterol is accounted for by the process of formation of myelin, by Schwann cells and oligodendroglia, respectively (11).

Our experimental design differs from the first of those described above (4). In that study the source of cholesterol was discussed in terms of the sterol balance in adult animals. Ours is a developmental paradigm so that we could directly compare the amount of cholesterol accumulated in an organ with that newly synthesized. Also, the methodology of the experiment is non-invasive, with the minor exception of the intraperitoneal injection of [^{3}H]water.

Our experimental design differs from the second of those described above (13) in that we evaluated small developmental increments and did not need to artificially rear animals. Also, we labeled biosynthesized cholesterol rather than that entering from the diet. Thus, our design extends past the period of weaning, allowing for simple dietary manipulation.

We acknowledge a number of weaknesses of our experimental design with respect to interpretation of radioactivity in cholesterol as an absolute amount of newly synthesized cholesterol. For example, the influence that age may have on the number of hydrogens that enter cholesterol from water was not evaluated in this study. This is relevant because the number 22 for ^{3}H atoms incorporated from [^{3}H]water may vary somewhat depending on how rapidly NADPH hydrogens are equilibrating with water; this varies depending on metabolic reliance on the pentose shunt (14). This cannot, however, be a serious error since the number 22 is close to the upper limit (1). A lower figure of 14.5 (15) does not seem feasible in this situation as this would result in a conclusion that nerve is synthesizing much more cholesterol than it is accumulating, an unlikely proposition. Another source of uncertainty is the calculation of the correction factor, L, of equation 1, which might also vary somewhat with age. We note, however, that our conclusions are largely based on a comparison of different tissues rather than relying only on absolute values, and at multiple age points. Thus, even quite significant errors in the above values should not alter our qualitative conclusions.

A conclusion we obtain from our data is that, during the course of development from birth to 35 days of age, almost all of the cholesterol accumulating in sciatic nerve has been synthesized in that tissue. A related observation reached in a different study (16) is that drug-induced lowering of circulating cholesterol in adult rats did not cause up-regulation of hydroxymethyl glutaryl-CoA reductase, the rate limiting enzyme of cholesterol biosynthesis, in sciatic nerve. Our conclusion is in contrast to the view that circulating cholesterol is in relatively rapid equilibrium with cholesterol in sciatic nerve myelin, and therefore might serve as the cholesterol source. Support for the latter mechanism is on the basis of experiments involving autoradiography of sciatic nerve after intraperitoneal injection of radioactive cholesterol; label quickly entered myelin (17). In retrospect, because such experiments could not be quantitated, those observations probably represented an equilibration of a minor fraction of injected cholesterol rather than representing a net transfer process.

Our interest in cholesterol in nerve derives from our studies that show peripheral demyelination, induced in weanling rats by tellurium exposure, occurs because a tellurium metabolite inhibits squalene epoxidase and thus blocks cholesterol synthesis (18). The lack of cholesterol, a major component of myelin, leads to destabililization and disintegration of the myelin sheath. The question arose as to why, in the face of what is a systemic inhibition of squalene epoxidase, the nerve is preferentially affected. The brain is spared because, even though it also produces cholesterol for synthesis of myelin, it does so at a rate more than an order of magnitude slower than does sciatic nerve (18). Thus, the deficit of cholesterol in brain is relatively less severe on a per unit weight basis and does not result in frank demyelination. Relevant to the situation in other tissues is the demonstration that in response to tellurium challenge, the cholesterol biosynthetic pathway in liver is up-regulated (19). Thus, many organs that can obtain cholesterol from the circulation should be unaffected. In contrast, the data presented herein, interpreted as demonstrating lack of utilization of circulating cholesterol by nerve, are compatible with the preferential susceptibility of nerve myelin formation to the tellurium insult.

We interpret our data for kidney as indicating primarily utilization of serum cholesterol during the suckling

period. Post-weaning, there is an increase in synthesis of cholesterol by kidney to a level accounting for over 50% of that accumulating. Unexpectedly, this increased synthesis is promoted by a cholesterol-free diet so that under such conditions all the cholesterol accumulating is locally synthesized. We note that our interpretation is in the context of cholesterol accumulation being related to kidney growth. A more complicated interpretation in terms of kidney being actively involved in cholesterol export could be considered (20). Our conclusion, that cholesterol synthesis by kidney may respond to diet in the same way as liver, is somewhat unexpected. We had assumed that, in extrahepatic organs, local synthesis of cholesterol would not be affected by diet. The level of circulating cholesterol (at least of unesterified cholesterol) does not seem to vary post-weaning as a function of cholesterol in the diet (Fig. 3). Thus, there is no obvious reason why various organs could not extract the same amount of cholesterol from the serum, irrespective of dietary cholesterol. Our results hint at the possibility that cholesterol synthesis by kidney is partially under hormonal or other systemic-level control. In considering experimental designs to further evaluate this question, it should be noted that dietary intervention does not alter circulating cholesterol levels and is thus different from designs using surgical or pharmacological techniques that perturb circulating cholesterol levels and may also introduce hormonal stress responses.

Our experimental design is applicable to other tissues and other structural components of cells, for example, analysis of the contribution of circulating free or esterified fatty acids to the pool supplying tissue fatty acids for formation of structural phospholipids.

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