Mode of Cholesterol Accumulation in Various Tissues of Rabbits with Various Serum Cholesterol Levels[1] (36758)

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Induction of hypercholesteremia by feeding a diet rich in cholesterol is a powerful means of producing atherosclerosis in susceptible animals (1). Elevated serum cholesterol in man is, indeed, associated with high incidence of coronary heart disease (2). The susceptibility of cholesterol deposition seems to vary from organ to organ. In animals resistant to atherosclerosis, such as dogs and rats, moderate elevation of serum cholesterol levels can only increase the cholesterol content of the liver but not other organs (3). In man, the arterial system is obviously the most susceptible organ for cholesterol accumulation, and, interestingly, is not accompanied by a simultaneous increase of the cholesterol contents of most other tissues in the body (4). Extremely high serum cholesterol levels, on the other hand, can result in accumulation of excessive cholesterol in various tissues or organs at least in certain species of animals (3, 5).

The three variables that are involved in this deposition in tissues are serum cholesterol level, tissue cholesterol content and the time factor. Their relationships can be evaluated by a factorial experiment or by considering two factors at a time with the third variable fixed at a constant value. The study (6) reported previously was designed to study the rate of cholesterol accumulation in various tissues of rabbits during a 12 wk period, with their serum cholesterol levels fixed in a narrow range of 300 to 400 mg/100 ml. Linear arithmetical increase of tissue cholesterol contents were found in adrenal, kidney, small intestine, and lung, while aorta, liver, spleen, and skin exhibited an exponential accumulation of cholesterol. The changes of cholesterol contents of other organs were much less conspicuous.

In order to evaluate the relationship between serum cholesterol levels and tissue cholesterol contents, 21 New Zealand white rabbits were employed in the present study. The serum cholesterol of the individual animals was fixed at a certain constant level which ranged from 30 to 2500 mg/100 ml among the groups. All animals were sacrificed at the same time—60 days after establishment of their fixed serum cholesterol levels.

Materials and Methods. Twenty-one New Zealand white adult male rabbits, weighing at the beginning of the experiment an average of 3200 g, were kept in individual cages in a well-ventilated animal facility. A 2% cholesterol diet was prepared by dissolving proper amounts of crystalline cholesterol in heated corn oil and mixing thoroughly with Rockland rabbit pellets. Each rabbit was fed, *ad libitum*, a mixture of normal Rockland rabbit pellets and the 2% cholesterol diet in a fixed proportion. The proportion varied from animal to animal and ranged from regular Rockland rabbit pellets to a 2% cholesterol diet. The purpose was to obtain a wide spectrum of serum cholesterol levels among these 21 rabbits. The desired levels of serum cholesterol in individual rabbits were usually established within 1 wk of cholesterol feed-

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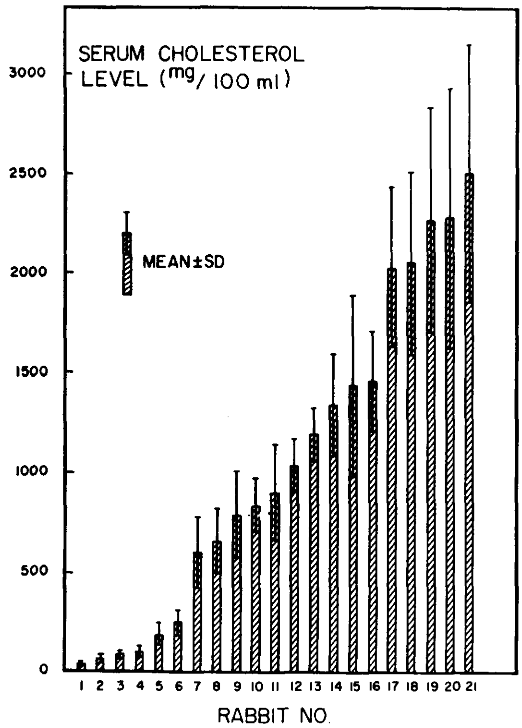

FIG. 1. Mean seurm cholesterol levels of individual rabbits with their standard deviations of 16 determinations over a period of 60 days.

ing. The serum cholesterol levels of each individual rabbit were thereafter kept as constant as possible throughout the experimental period by (a) checking their serum cholesterol levels every 3 to 4 days and (b) constant adjustment of their diets by either increases or decreases of the cholesterol contents. The serum cholesterol levels were determined by autoanalyzer method employing Liebermann–Burchard color reagent (7, 8). All animals were sacrificed by exsanguination through the abdominal aorta 60 days after establishment of their fixed serum cholesterol levels. The cholesterol contents of various tissues, namely skin, aorta, spleen, lung, liver, adrenal, testis, kidney, colon, small intestine, heart, pancreas, adipose tissue, skeletal muscle and brain, were determined by the method described previously (3).

Results. The mean serum cholesterol levels of individual rabbits with their standard deviations of 16 determinations over a period of 60 days are graphically shown in Fig. 1. The means of individual serum cholesterol levels fell in a wide spectrum, ranging from 31 to 2486 mg/100 ml. There were also considerable individual variations as reflected by the large standard deviations in some animals. Nevertheless, the individual mean serum cholesterol levels were used for regression analysis.

The regression analysis was carried out between the serum cholesterol levels (100 mg/100 ml) and the cholesterol contents of a particular tissue (g of cholesterol/100 g of dry tissue). The analysis was done in both arithmetical scales and logarithmic scales for tissue cholesterol contents. The logarithmic scales of the tissue cholesterol contents were adopted only when the regression analysis revealed (a) a much better correlation coefficient, (b) smaller standard deviation of estimate (SEE), and (c) the value of Y-intercept closer to the control value than those when arithmetical scales were used. The better fit of the logarithmically transformed values of tissue cholesterol contents in regression analysis indicated an exponential rather than an arithmetical linear relationship between these two variables, *i.e.*, tissue cholesterol contents and serum cholesterol levels. Among these 15 tissues, skin, aorta, spleen and lung showed exponential increases of their cholesterol contents with increases in their serum cholesterol levels, whereas most other tissues exhibited only an arithmetical linear increase. Muscle and brain were the only two tissues having a constant cholesterol content in spite of the changes in serum cholesterol levels. Therefore, the tissues or organs in rabbits could be grouped into the following three categories according to their response to various degrees of hypercholesteremia:

Group A. Exponential increase of tissue cholesterol contents with elevation of serum cholesterol levels (Fig. 2). Such relationship was found in skin, aorta, spleen and lung. The slopes of the regression lines shown in Fig. 2 represent the increment of tissue cholesterol (in logarithmic scale) for 1 unit (100 mg/100 ml) elevation of serum cholesterol levels. Such increment was largest for skin (0.156), then aorta (0.130), and spleen

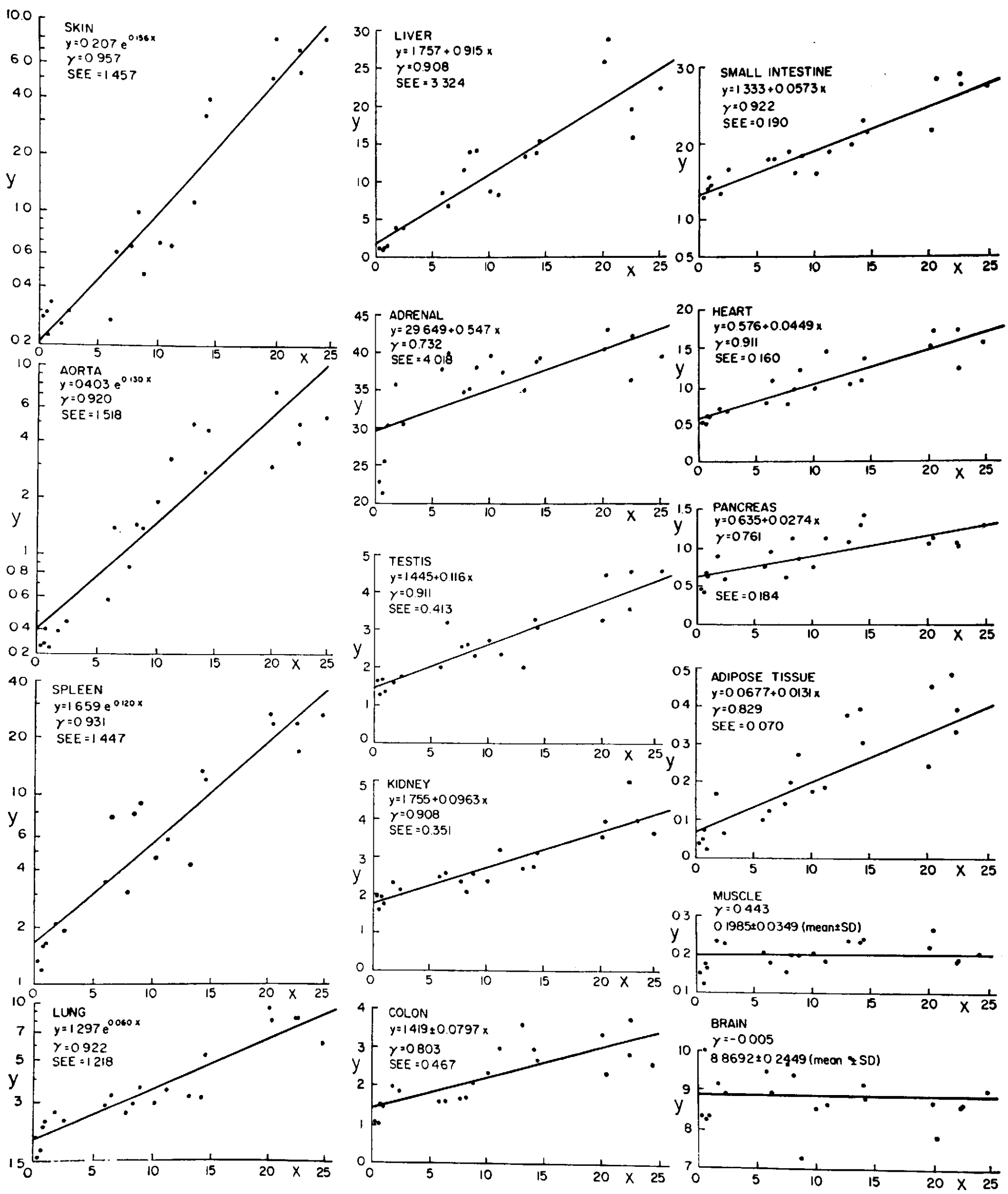

FIG. 2. Regression analysis of cholesterol content of 15 tissues (g cholesterol/100 g dry tissue) with serum cholesterol levels (100 mg cholesterol/100 ml). The latter of individual rabbits was kept constant for 60 days. y = grams of cholesterol per 100 g of dry tissue in logarithmic scale; (middle and right column) y = grams of cholesterol per 100 g of dry tissue in arithmetical scale. x = serum cholesterol level in 100 mg/100 ml; (left column).

(0.120), and smallest for lung (0.060).

Group B. Linear arithmetical increase (Fig. 2). Nine organs were included in this category. In the order of decreasing magnitudes of increments, they were liver (increment or slope of regression equation = 0.915 g/100 g of dry tissue for each increase of 100 mg/100 ml of serum cholesterol), adrenal (0.547), testis (0.116), kidney (0.0963), colon (.0797), small intestine (0.0573), heart (0.0449), pancreas (0.0274), and adipose tissue (0.0131).

Group C. No significant change in tissue cholesterol contents (Fig. 2). Muscle and brain belonged in this category. The cholesterol contents of muscle and brain were 0.1985 ± 0.0349 g/100 g of dry tissue (mean ± SD) and 8.8692 ± 0.2449 g/100 g of dry tissue, respectively; they were not significantly affected by changes in serum cholesterol levels.

Discussion. Tissues with little or no changes in cholesterol contents. In our previous study, the serum cholesterol levels of 12 rabbits were kept between 300 and 400 mg/100 ml for a period of 12 wk. All tissues except brain, skeletal muscle, adipose tissue and pancreas increased their cholesterol contents, whereas in the present study, adipose tissue and pancreas also showed a significant increase of their cholesterol contents when the serum cholesterol was elevated beyond 400 mg/100 ml for 60 days, although the increments were less conspicuous than those of other tissues. The cholesterol contents of brain and skeletal muscle did not increase under these conditions. We have previously shown that the muscle of rabbits fed a 2% cholesterol diet for 3 months did not show any change in its cholesterol content, but, indeed, increased its cholesterol content 3- to 4-fold at the end of 9 mo of cholesterol feeding (3). Therefore, there was a definite variation of the susceptibility of different tissues to cholesterol deposition. The cholesterol content in the brain was constant in all studies. The resistance of brain tissue to excessive cholesterol deposition might be due both to (a) the blood–brain barrier which blocks the passage of cholesterol from the vascular system into the brain parenchyma (9), and to (b) the slow turnover of the structural cholesterol in the adult brain (10). Skeletal muscle seems to be the second most resistant tissue to cholesterol deposition which occurred only after prolonged exposure to extreme hypercholesteremia (3). The thresholds for cholesterol deposition in adipose tissue and pancreas were apparently higher than those of other tissues (except brain and muscle). Since muscle and adipose tissue are two major tissues in the body, their resistance to accumulation of excessive cholesterol in a moderately hypercholesteremic state may partially explain the fact that the total body exchangeable cholesterol pool was not greatly expanded under such conditions (11).

Tissues with linear arithmetical increases. In our previous study testis, colon and heart showed random increases in their cholesterol contents with no particular relation to the duration of exposure to moderate hypercholesteremia (6), but in the present study, they did increase proportionally with the increase of the levels of serum cholesterol. It seems to be the level of serum cholesterol, but not the time factor, which was more important in determination of the extent of cholesterol accumulation in these three tissues.

Most tissues or organs in rabbits increased their cholesterol contents in a linear arithmetical proportion to the serum cholesterol levels. It is the net influx of cholesterol into the particular tissue which is proportional to the serum cholesterol levels. The net influx is the difference between total influx and total efflux. The latter might be negligibly minute, a fixed rate, or a fixed percentage of the total influx. After 60 days of exposure to various serum cholesterol levels, the capacities of all these tissues to accumulate cholesterol have obviously not been totally saturated since their regression lines have not reached a plateau. Among these tissues, liver is the one considered to be in the same pool as the blood. The fact that the largest increment was found in the liver indicates that the liver has an enormous capacity and affinity for circulating cholesterol and may play an important role in the regulation of its levels.

Tissues with exponential increases. The exponential increase of tissue cholesterol contents with elevation of serum cholesterol levels found in skin, aorta, spleen and lung is more complicated than the straightforward linear arithmetical changes. The simple translation of such mathematical terms is that the deposited cholesterol in such tissue in duces or facilitates more cholesterol deposition; it could be compared with the growth of a bank deposit at a certain percent of interest compounded daily. In other words, a vicious cycle has been created by the initial overinflux of serum cholesterol into the tissue. The nature of this vicious cycle is still unsettled, but there are two possible mechanisms:

1. Cholesterol feeding has been shown to damage intimal cells and to induce proliferation of smooth muscle cells or multifunctional mesenchymal cells in the aortic wall which are then transformed into foam cells with histiocytic characteristics (12). The foam cells found in atheromata of skin are also of histiocytic origin. Spleen and lung are also rich in reticuloendothelial elements. The influx of cholesterol from blood to such tissues may stimulate the activity of the reticuloedothelial system, mainly proliferation of histocytes, which will phagocytize more cholesterol and lipid. The accumulation of more cholesterol in turn stimulates more cellular proliferation, resulting in a vicious cycle until the tissue capacity is totally saturated. Liver is also a major reticuloendothelial organ. However, the cholesterol deposition is not just limited to von Kupffer's cells, but also involves hepatic cells. As a matter of fact, the results obtained from liver can fit, equally well, both a linear arithmetical increase as an exponential increase.

2. Cholesterol feeding has also been shown to increase the permeability of the vascular wall; this allows more cholesterol to deposit in the tissue and initiates the vicious cycle discussed previously (13).

Skin and aorta are the two organs showing the greatest affinity to the circulating cholesterol in the form of lipoproteins. Such a phenomenon has also been observed in prairie dogs (3) and in rhesus monkeys (14, 15), and is most likely true in humans too since extreme hypercholesteremia in man is invariably associated with cutaneous xanthomatosis and severe atherosclerosis (16).

Factorial experiment needed. Our previous (6) and present studies thus provided evidence of tissue variation in the susceptibility to deposition of excessive cholesterol. However, these studies only analyze two variables with the third variable fixed at a particular value, in each of two studies: the earlier study analyzed tissue cholesterol contents with the duration of exposure to a fixed serum cholesterol level between 300 and 400 mg/100 ml up to 12 wk; the present study analyzed tissue cholesterol contents with serum cholesterol levels from 31 to 2486 mg/100 ml for a fixed period of 60 days.

In order to be able to view the whole picture and also to predict the cholesterol content of any given tissue with a given serum cholesterol level for a given time, a factorial experiment involving all three variables at the same time (tissue cholesterol content, serum cholesterol level, and duration of exposure) should be carried out.

Summary. The individual serum cholesterol of 21 adult male New Zealand white rabbits were maintained constant at certain fixed levels ranging from 31 to 2486 mg/100 ml among the group for 60 days by frequent adjustment of their dietary cholesterol intake. The cholesterol contents of 15 different tissues of all rabbits were determined at the end of the 60 day experiment. Brain and muscle did not show any significant change in their cholesterol contents. The cholesterol contents of most tissues including liver, adrenal, testis, kidney, colon, small intestine, heart, pancreas and adipose tissue increased linearly in proportion to serum cholesterol levels, whereas that of skin, aorta, spleen and lung increased exponentially with the elevation of serum cholesterol levels. The study thus provided evidence of variations in the susceptibility of different tissues to deposition of excessive cholesterol.

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