Mode of Cholesterol Accumulation in Various Tissues of Rabbits with Moderate Hypercholesteremia[1] (35238)

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Beaumont *et al.* (1) showed a steady increase of cholesterol in several tissues in rabbits fed a 1% cholesterol diet for 4 months. Ho and Taylor (2) also reported that following 1 year of feeding of a 2% cholesterol diet to rabbits, a variable degree of increase in cholesterol content was found in all tissues except the brain. There are three parameters which should be considered in these studies, *i.e.*, tissue cholesterol content, time factor, and plasma cholesterol level. One may ask, what are the relationships among these three variables? It is difficult to evaluate these relationships from the studies mentioned previously since the serum cholesterol levels varied from time to time in the individual animals. It would be easier to find out these relationships by considering two factors at a time and carrying out two separate studies:

1. Keep the plasma cholesterol of all the animals constant at the same levels and study the correlation between tissue cholesterol content and the duration of time.

2. Keep the plasma cholesterol constant in individual animals but at various levels in different animals over the same period of time and study the correlation between cholesterol content and the plasma cholesterol levels. A combination of the results obtained from (1) and (2) by a multiple regression study may reveal the true complicated relationships among these three parameters. This report concerns the first part of these studies, namely the study of the correlation of cholesterol content of various tissue with the duration of moderate hypercholesteremia in the rabbit.

[1] This work was supported by a U.S. Public Health Service Grant (HE-11029-4) from the National Heart Institute.

Materials and Methods. Twenty New Zealand white adult male rabbits, weighing an average of 2550 g, were used in the experiment. The rabbits were kept in individual cages in a well-ventilated animal facility. The rabbit diet, containing 0.25, 0.4, 1.0, and 2% of cholesterol, was prepared by dissolving proper amounts of crystalline cholesterol in heated corn oil and mixing thoroughly with Rockland rabbit pellets. All rabbits were fed a 2% cholesterol diet for the first 3 days, and subsequently they were fed diets with different percentages of cholesterol, depending upon their plasma cholesterol levels which were determined by the method of Abell *et al.* (3). The plasma cholesterol levels thus were kept constant (between 300–400 mg/100 ml) by weekly determinations of plasma cholesterol and adjustment of dietary cholesterol. Eight rabbits were excluded from the study because their weekly plasma cholesterol levels were found once or more than once to be either below 300 mg/100 ml or above 400 mg/100 ml. The rest of rabbits were killed one every week up to 12 weeks. The cholesterol content in various tissues (namely aorta, liver, spleen, skin, adrenal, kidney, small intestine, lung, testis, colon, heart, muscle, fat, brain, and pancreas) was determined by the method described previously (2).

Results. The plasma cholesterol levels of the 12 rabbits in the study were tested weekly throughout the entire experimental period and were all within the range of 300 to 400 mg/100 ml with an average of 347 mg/100 ml. The tissue cholesterol concentration was expressed as grams of cholesterol per 100 g of dry tissue. The cholesterol contents of the same kinds of tissues or organs of the 12 rabbits sacrificed at different times over a

TABLE I. Correlation of Cholesterol Contents of Various Tissues with Duration of Moderate Hypercholesteremia (300 to 400 mg/100 ml) in Rabbits.[a]

	Tissue	γ	$\frac{\gamma}{SEr}$	p	Syx	Regression equation
A	Aorta	0.883	2.923	0.003	0.070	$Y = 0.436\ e^{0.201x}$
	Liver	0.834	2.752	0.005	0.384	$Y = 1.240\ e^{0.170x}$
	Spleen	0.782	2.580	0.007	0.306	$Y = 1.70\ e^{0.145x}$
	Skin	0.840	2.772	0.005	0.279	$Y = 0.292\ e^{0.126x}$
B	Adrenal	0.835	2.855	0.004	3.369	$Y = 25.21 + 1.49x$
	Kidney	0.654	2.158	0.032	0.269	$Y = 1.73 + 0.065x$
	Small intestine	0.834	2.752	0.005	0.214	$Y = 1.40 + 0.094x$
	Lung	0.642	2.119	0.034	0.189	$Y = 2.71 + 0.045x$
C	Testis	0.453	1.504	0.13	0.333	
	Colon	0.479	1.589	0.11	0.318	
	Heart	0.279	0.927	0.32	0.155	
D	Muscle	0.481	1.596	0.10	0.048	
	Fat	0.346	1.148	0.22	0.034	
	Brain	0.345	1.145	0.23	0.704	
	Pancreas	0.084	0.280	0.76	0.179	

[a] γ = correlation coefficient; SEr = standard error of r; p = probability or significant level; Syx = standard error of estimate; Regression equation: Y: cholesterol content of tissue (g/100 g of dry tissue); X: time in weeks; e: base of natural logarithm.

period of 12 weeks were used for a study of their correlation with the time course. The results are summarized in Table I.

Eight of 15 tissues studied, *i.e.*, aorta, liver, spleen, skin, adrenal, kidney, small intestine, and lung showed significant increases of tissue cholesterol content with increase in the time of exposure to moderate hypercholesteremia. Moreover, the first four tissues, *i.e.*, aorta, liver, spleen, and skin exhibited an exponential increase of their tissue cholesterol contents; whereas the last four tissues, *i.e.*, adrenal, kidney, small intestine, and lung had only an arithmetical linear correlation. The rest of tissues, *i.e.*, testis, colon, heart, muscle, fat, brain, and pancreas, however, did not reveal a similar significant correlation. Although testis, colon, and heart did indeed increase their cholesterol content after cholesterol feeding, such increases were so random that no significant correlation with time course could be found. No increase of tissue cholesterol content was found in muscle, fat, brain, and pancreas. Therefore, the tissues or organs in rabbits could be grouped into four categories according to their response to dietary induced moderate hypercholesteremia:

Group A. Exponential increase of tissue cholesterol with increase of time as shown in Table I and Fig. 1. Such organs showed the fastest increase in their cholesterol content after cholesterol feeding. Among them, the increment was largest for aorta (0.201), then liver (0.170), and spleen (0.145), and smallest for skin (0.126).

Group B. Linear arithmetical increase as shown in Table I and Fig. 2. The increment was rather small for kidney, small intestine, and lung but large for adrenal.

Group C. Random increase with no particular relation to time. Such organs included testis, colon, and heart (Table I). Their increments were usually small and irregular.

Group D. No significant change in tissue cholesterol content. Such organs included muscle, fat, pancreas, and brain (Table I).

Discussion. The rabbit is well known to be very susceptible to development of hypercholesteremia after cholesterol feeding since this animal lacks of efficient homeostatic protective mechanisms such as a significant limitation of intestinal absorption of dietary cholesterol, marked suppression of en-

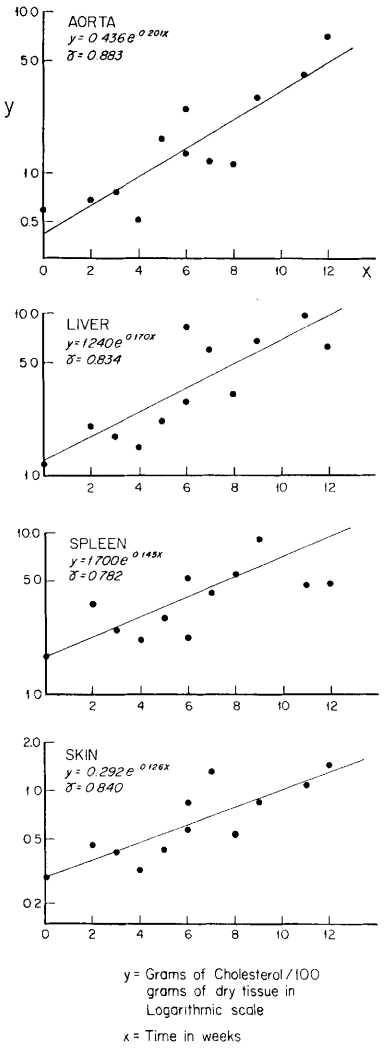

FIG. 1. Exponential accumulation of cholesterol in aorta, liver, spleen, and skin.

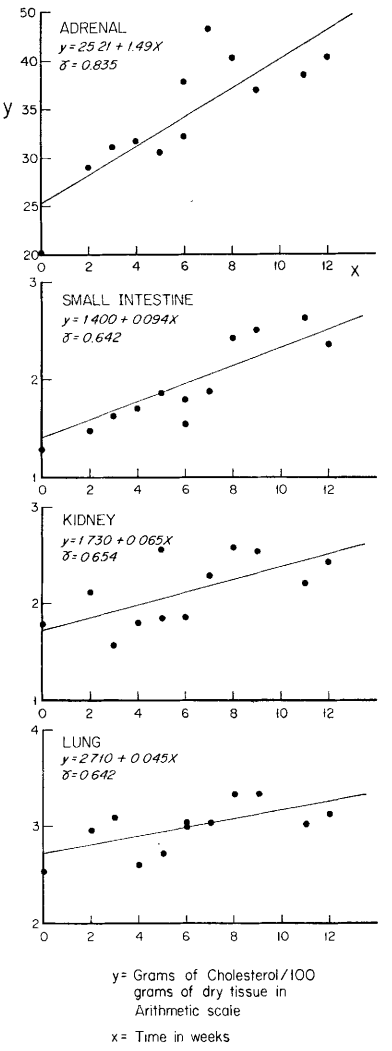

FIG. 2. Linear arithmetic increase of cholesterol in adrenal, small intestine, kidney, and lung.

dogenous cholesterol synthesis and increase of fecal excretion of neutral and acid steroids (4). Cholesterol is poured constantly from intestine into plasma and finally accumulated in the tissues. Previous studies showed that most organs reached maximal saturation in a short period of time when exposed to extreme hypercholesteremia (2). This may well represent experimental conditions that are too rapid and overwhelming for a proper study of the pattern or mechanism of cholesterol accumulation in tissues. Therefore, only moderately high plasma cholesterol levels were induced and maintained in this study.

The linear arithmetical increase of cholesterol content in adrenal, kidney, small intestine, and lung is easily understood if one visualizes that when the plasma cholesterol levels were constant and the tissue capacity for uptake of cholesterol was far below saturation, then there would be a constant net amount of cholesterol accumulated in the tissue. However, the mechanism of exponential increase of cholesterol content in aorta, liver, spleen, and skin is probably more complicated. There are two possible explanations for this: The first explanation is that the influx of cholesterol from blood to certain tissues

may stimulate the activity of the reticuloendothelial system. Liver and spleen are major reticuloendothelial systems in the body. The foam cells found in skin are also of histiocytic origin. While the foam cells in the aorta are considered to be transformed smooth muscle cells or multifunctional mesenchymal cells (5), they also have histiocytic characteristics. Thus, the stimulation of proliferation of these phagocytic cells may result in more accumulation of cholesterol in those tissues which in turn stimulates more proliferation and results in a vicious cycle until the tissue capacity is totally saturated.

The other explanation is that the early accumulation of cholesterol in the tissue may damage the tissue resulting in an increase of permeability of vascular walls with a resultant increased deposition of cholesterol in extravascular compartments.

The greatest increment of cholesterol was found in the aorta. This phenomenon was also found in prairie dogs (2) and rhesus monkeys (6). The removal of excessive cholesterol from aorta of rabbits after cessation of cholesterol feeding has been shown to be extremely slow (2). The rapid and avid uptake of cholesterol and its slow removal is a unique characteristic of aorta and may play an important role in the pathogenesis of atherosclerosis.

Summary. A moderate hypercholesteremia (300 to 400 mg/100 ml) was induced and maintained in a group of New Zealand white rabbits. The animals were killed weekly up to 12 weeks and cholesterol contents of 15 tissues were determined. Muscle, fat, pancreases, and brain showed no increase of their cholesterol contents. Small increases were noted in testis, colon, and heart, but these were not related to time. Linear arithmetic increases 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 over a 3-month period of moderate hypercholesteremia. The rapid and avid uptake of cholesterol by aorta may play a role in the pathogenesis of atherosclerosis.

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Received Sept. 1, 1970. P.S.E.B.M., 1971, Vol. 136.