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Independence of arterial phospholipid synthesis from alterations in blood lipids*

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SUMMARY

Rabbits fed cholesterol for 1 to 5 months showed gradually increasing amounts of aortic phospholipid preceded by increases in plasma phospholipid concentration. Cholesterol feeding appeared to increase the turnover of plasma as well as aortic phospholipid. Curtailment of plasma phospholipid synthesis to one-tenth of normal by evisceration did not diminish the incorporation of P^{32} into aortic lipids. In these animals the aortic phospholipid specific activity was 15 to 90 times as great as that of plasma, so that practically all the labeled aortic phospholipid must have been synthesized *in situ*. Lowering the plasma lipid levels by removal of cholesterol from the diet did not diminish the cholesterol content of the aortic lesion or alter its phospholipogenesis.

In previous investigations we have shown that in the cholesterol-fed rabbit, phospholipids accumulate in aortic plaques, and that these phospholipids were probably not deposited from plasma but instead were formed by synthesis in the arterial wall (1). This evidence was obtained by the injection of P^{32}-phosphate and P^{32}-labeled lipoproteins, which demonstrated that over 90 per cent of the radioactive phospholipid in the arterial wall was derived from synthesis. This conclusion was confirmed by the observation that early after P^{32}-phosphate administration, the specific activity of three different phospholipid fractions was greater in the artery than the time-average specific activity of the same phospholipids in plasma (2). Several questions remained to be answered, however. According to our concept that the artery is the source of plaque phospholipids, one should be able to alter the specific activity of the plasma phospholipids drastically without noticeable effect on the incorporation of P^{32} into arterial phospholipids. This was accomplished in the experiments to be reported here by evisceration of the animals prior to P^{32} administration. After demonstrating that the incorporation of P^{32}-phosphate into arterial phospholipid was independent of the level of radioactive plasma phospholipids, it seemed important to find out whether this incorporation would depend on the concentration of nonradioactive lipids in the blood. This question was studied in rabbits with atheromatous lesions and normal blood lipid levels. These studies indicated that the rate of phospholipid synthesis from P^{32} in the atheromatous aorta did not decrease when the plasma lipid levels were lowered. It thus appears that arterial phospholipid synthesis is a process closely related to, if not a part of, the development of experimental atherosclerosis and that the regulation of the synthetic rate is not a simple function of blood lipid concentration.

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EXPERIMENTAL

Experimental atheromatosis was produced in New Zealand white rabbits by daily feeding of 100 g. Purina rabbit chow containing 1 g. cholesterol and 2.6 g. fat.[1] Control animals, which received the same amount of rabbit chow per day, were chosen at the beginning of all feeding periods and were paired with respect to age and source.

Rabbits were injected with 0.5 mc. P^{32}-phosphate and usually plasma, liver, and aorta were taken 4 to 6 hours later for phospholipid P^{31} and P^{32} determination. Some rabbits were killed 6, 24, and 48 hours after P^{32} administration for determination of specific activities of phospholipids in plasma and aorta at various time intervals. To obtain measures of acid-soluble phosphate specific activity in the latter experiment, tissues were first extracted with trichloroacetic acid

[1] Kopald vegetable fat, Humko Co., Memphis, Tenn.

TABLE 1. CONCENTRATION OF PHOSPHOLIPIDS AFTER VARIOUS INTERVALS OF CHOLESTEROL FEEDING

Months	Aorta		Plasma		Liver	
	Control Diet	Cholesterol Diet	Control Diet	Cholesterol Diet	Control Diet	Cholesterol Diet
1	0.160 ± 0.043	0.189 ± 0.059	0.0388 ± 0.0042	0.281 ± 0.071	1.01 ± 0.09	0.889 ± 0.092
2	0.177 ± 0.012	0.372 ± 0.080	0.0442 ± 0.0074	0.252 ± 0.033	1.00 ± 0.12	1.07 ± 0.04
3	0.172 ± 0.022	0.663 ± 0.121	0.0414 ± 0.0068	0.349 ± 0.038	1.30 ± 0.09	1.33 ± 0.06
5	0.147 ± 0.041	0.520 ± 0.046	0.0468 ± 0.0042	0.330 ± 0.031	1.12 ± 0.01	0.995 ± 0.10

Concentrations are expressed as mg. phospholipid P/cc. plasma or/g. fresh tissue. Mean of 3 animals ± standard error.

and then with chloroform-methanol for lipid analysis. Therefore the absolute lipid values in this experiment differed slightly from those obtained when trichloroacetic acid was not used.

In all experiments extraction of tissue lipids was carried out by the method of Folch *et al.* (3). Phospholipid phosphorus in the purified extracts was determined by the method of King (4). Cholesterol was determined on the same extract after saponification and extraction with petroleum ether. To a dried aliquot was added 4 ml. of a solution containing 2.9 per cent p-toluenesulfonic acid, 25.7 per cent glacial acetic acid, and 71.4 per cent acetic anhydride; 30 minutes after addition of 0.4 ml. concentrated H_2SO_4, the optical density was determined at 550 mμ. (5).

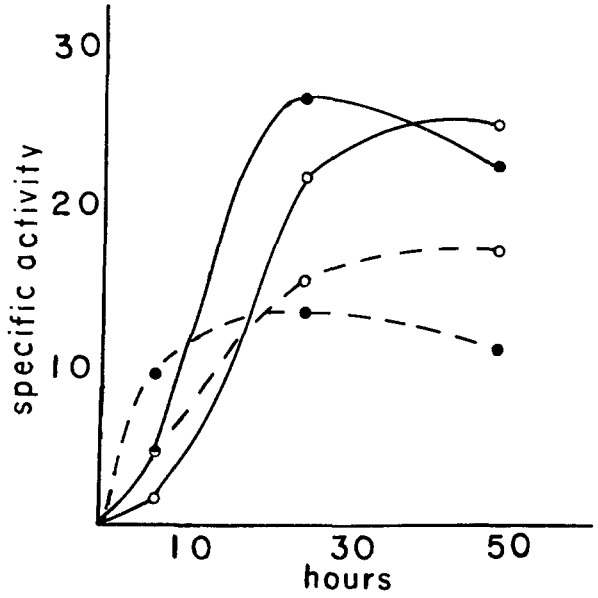

FIG. 1. Specific activity-time relations of plasma (——) and aortic (– – –) phospholipid in control (●) and cholesterol-fed (○) rabbits. Specific activities are expressed as per cent of injected P^{32} per g. of phospholipid phosphorus. Each point represents the mean obtained on 3 animals.

RESULTS

A prerequisite for quantitative isotope studies is the knowledge of specific activity-time relations of the material under study and, if possible, of its immediate precursor. Although some data obtained in our laboratory showed that 6 hours after P^{32} administration the specific activity of aortic phospholipid was still increasing, no literature is available to indicate these specific activity-time relations. Figure 1 shows specific activities of plasma and aortic phospholipids at various times after the injection of P^{32}-phosphate in control and cholesterol-fed rabbits (1 g. cholesterol per day for 5 months). Each point represents the average of duplicate determinations on each of three animals. Although the small number of animals employed per time interval does not allow a detailed interpretation of differences in the specific activity-time course of control and cholesterol-fed rabbits, the data are sufficient to illustrate the following points: (*a*) at the 6-hour interval used in previous studies (6) the specific activity of aortic phospholipid exceeds that in plasma; (*b*) at later intervals the specific activity of plasma rises so rapidly that it soon exceeds that of the aorta; (*c*) the aortic specific activity continues to rise and does not level off until about 24 hours after P^{32} injection. The choice of the 6-hour time interval between injection of P^{32} and killing the animal appears to be appropriate since at this time the specific activity of the plasma still lags behind that of the aorta. This is advantageous for the detection of phospholipid synthesis by the aorta in the presence of a large pool of plasma phospholipid in immediate contact with the artery.

The present study was also concerned with the sequence of phospholipid alterations in plasma and aorta at different intervals after the beginning of a cholesterol-rich diet. Two questions appeared to be of impor-

tance: (*a*) would the increase in arterial plaque phospholipid be preceded by an elevation of plasma phospholipid, and (*b*) would one be able to detect by the P^{32} technique early changes in arterial phospholipid metabolism before any histological or chemical alterations were evident? To study these questions, four groups of three cholesterol-fed and three control rabbits were sacrificed 1, 2, 3, and 5 months after the beginning of the diets. Six hours prior to the terminal samples, 0.5 mc. P^{32}-phosphate was administered intravenously. Table 1 presents phospholipid concentrations in aorta, plasma, and liver in these animals. In confirmation of earlier work (7) it was observed that although the liver was quite fatty, the liver phospholipid concentration per g. of fresh tissue did not increase during the 5-month period. In plasma, major increases in phospholipid concentration became evident after 1 month and in the aorta after 2 months, when the cholesterol-fed animals were compared to their own controls. A study of the plasma and arterial phospholipid concentrations in individual animals revealed that plasma phospholipids rose before any increase in arterial phospholipids occurred. Weinhouse and Hirsch (8) similarly observed that the cholesterol in plasma increases before that in the aorta. This observation and the finding that the lipid partition of the fresh plaque resembles that of the plasma (9) have contributed greatly to the conclusion that plaque lipids are derived from the circulating blood. The P^{32} data in Table 2, in confirmation of earlier observations (6), do not substantiate this conclusion for the phospholipid moiety of the plaque. In the cholesterol-fed animals the specific activity of the acid-soluble phosphates of plasma and aorta did not differ from the controls but the specific activity of arterial phospholipids greatly exceeded that of plasma. If for the moment we ignore the fact that neither the plasma nor the arterial phospholipids are homogeneous, a subject that has been dealt with in a previous publication (2), this difference in phospholipid specific activities would appear to rule out the possibility that a major portion of arterial phospholipids derives from plasma.

Two additional facts emerge from Table 2. The specific activities of phospholipid of control arteries, of arteries with minimal plaques (1 and 2 months) and of arteries with major lipid deposits (3 and 5 months) did not differ appreciably. This indicates that the per cent phospholipid renewed is constant. In other words, per unit of phospholipid complex, be it lipoprotein or a lipid micelle, the turnover is the same in the pathological state as in the normal condition. Since, however, the total thoracic aorta of the severely

TABLE 2. SPECIFIC ACTIVITIES OF PHOSPHOLIPIDS AFTER VARIOUS INTERVALS OF CHOLESTEROL FEEDING 6 HOURS AFTER P^{32} ADMINISTRATION

Months	Cholesterol Diet		Control Diet	
	Aorta	Plasma	Aorta	Plasma
1	6.69 ± 1.88	2.51 ± 0.05	5.84 ± 0.60	5.62 ± 1.64
2	7.65 ± 1.06	1.54 ± 0.19	6.55 ± 3.55	5.11 ± 0.76
3	8.94 ± 2.66	2.62 ± 0.87	7.29 ± 0.69	7.85 ± 2.57
5	7.57 ± 0.37	2.39 ± 0.38	7.24 ± 1.51	4.86 ± 0.69

Specific activities are expressed as per cent injected P^{32}/g. phospholipid P. Mean of 3 animals ± standard error.

atherosclerotic animal contains eight times as much phospholipid as the control artery [2] the total turnover rate of aortic phospholipid was increased by a factor of eight. The other fact which merits discussion concerns the plasma phospholipids. It is evident from Table 2 that the specific activity of plasma phospholipids in the cholesterol-fed animal is lower than in the normal control. One might attempt to translate the depressed plasma phospholipid specific activity in terms of a decrease in plasma phospholipid turnover rate but this would not take into account the fact that the phospholipid concentration of the plasma is increased several-fold. Thus a better measure of turnover rate of plasma phospholipid is the concentration of radioactive plasma phospholipid divided by the liver phospholipid specific activity, since the liver is the primary source of plasma phospholipid.[3] This average ratio is 0.020 ± 0.001 and 0.060 ± 0.004 respectively for the controls and cholesterol-fed rabbits and shows that in the latter the turnover rate of plasma phospholipids is actually increased ($p < 0.001$). One might conclude, therefore, that both plasma and arterial phospholipid turnover per whole organ is greatly increased in the atherosclerotic rabbit and that, as was the case for the concentrations, the increased turnover of plasma

[2] Although Table 1 shows only a fourfold increase in phospholipid concentration, it should be noted that the aorta of the cholesterol-fed animal weighs more than two times that of the control.

[3] Experiments have demonstrated (10) that in the rabbit the removal of the gastrointestinal tract, spleen, liver, and kidneys decreased plasma phospholipid synthesis to approximately one-tenth. Whether the assumption is made that liver is the primary source of plasma phospholipid, or that all of these tissues contribute, does not alter our basic finding of increased plasma phospholipid turnover, as liver, intestine, spleen, and kidney specific activities are of the same order of magnitude and are not altered by cholesterol feeding (7).

TABLE 3. SPECIFIC ACTIVITIES OF TISSUE PHOSPHOLIPIDS IN EVISCERATED-NEPHRECTOMIZED CHOLESTEROL-FED AND SHAM-OPERATED CHOLESTEROL-FED RABBITS 4 HOURS AFTER P^{32} ADMINISTRATION

	Eviscerated-Nephrectomized	Sham-Operated
Liver	0.0242 ± 0.0138	9.59 ± 1.12
Plasma	0.0659 ± 0.0208	0.580 ± 0.099
Intima	4.27 ± 0.68	4.58 ± 0.78
Residual Aorta	5.23 ± 0.56	3.55 ± 0.23

Specific activities are expressed as per cent injected P^{32}/g. phospholipid P. Mean of 3 animals ± standard error.

phospholipids precedes the increase in the artery. However, the isotope data do not support the thesis that plasma phospholipids function as precursors of arterial phospholipids. Unfortunately the P^{32} technique did not measurably increase the sensitivity of detection of early atherosclerotic changes in the arterial wall since changes in P^{32} concentrations did not precede, and were no greater than, changes in chemical phospholipid concentrations.

Although the above experiment confirmed previous evidence (1) that the majority of the arterial phospholipid was synthesized *in situ*, it might still be possible that a very small portion of arterial phospholipid was derived from deposition of blood phospholipid or blood lipoproteins. Therefore the incorporation of P^{32} into arterial phospholipid was studied in animals in which the formation of plasma P^{32}-phospholipids was severely depressed by the removal of gastrointestinal tract, spleen, and kidneys, and the occlusion of the hepatic artery (10). If most of the P^{32}-phospholipid in the atheromatous lesion is derived from synthesis in the artery, one should expect no diminution of arterial phospholipid P^{32} in the face of a greatly diminished plasma phospholipid P^{32}. The results of such an experiment are given in Table 3. In order to work with eviscerated animals before they deteriorated too much, the experiment was terminated 4 hours after injection of P^{32}-phosphate. The extent to which incorporation of P^{32} into liver phospholipids is depressed attests to the success of the hepatectomy. Apparently plasma phospholipid synthesis is practically stopped by evisceration in the cholesterol-fed animal, as in normal controls previously studied (10). The most significant finding was, however, that the incorporation of P^{32} into arterial phospholipids of eviscerated and sham-operated animals was practically the same, although the average plasma phospholipid P^{32} in the eviscerates was only one-tenth that of the sham-operated controls.[4] Stronger evidence that practically all phospholipid P^{32} came from arterial synthesis may be gleaned from a comparison of the terminal specific activities of aortic and plasma phospholipids in individual eviscerated cholesterol-fed rabbits, as in Table 4. In some of the eviscerates plasma phospholipid-P^{32} incorporation was not so greatly depressed as in others. In one animal, however, the ratio of aortic to terminal plasma phospholipid specific activity reached 93.8, which is clear evidence that the P^{32}-phospholipid was derived entirely from aortic synthesis.

From the above experiments no evidence could be obtained to suggest that deposition of plasma phospholipids is responsible for the incorporation of P^{32} into the phospholipids of arterial plaques. Yet the question whether plasma lipids are causally linked in some other manner to the development of arterial lesions cannot be resolved by these experiments. Perhaps a high concentration of plasma cholesterol or plasma phospholipid is required to bring about the accumulation of arterial phospholipids by means of an increase in phospholipid synthesis. We have attempted to answer this question by comparing the synthesis of phospholipids in atheromatous lesions of hyper- and normolipemic animals. The latter were prepared by feeding 1 g. of cholesterol per day for 3 months and subsequent removal of dietary cholesterol for 2 months. During the latter period the blood lipids de-

TABLE 4. COMPARISON OF TERMINAL PHOSPHOLIPID SPECIFIC ACTIVITIES IN INDIVIDUAL EVISCERATED-NEPHRECTOMIZED CHOLESTEROL-FED RABBITS

Aortic Intima	Plasma	Aortic Intima / Plasma
4.01	0.208	19.3
2.53	0.190	13.3
6.05	0.284	21.3
3.85	0.062	62.1
5.50	0.099	55.5
3.47	0.037	93.8

Specific activities are expressed as per cent injected P^{32}/g. phospholipid P.

[4] One might expect to find an increased specific activity in both lesions and residual aorta of the eviscerated-nephrectomized rabbit resulting from a decreased precursor pool which was reflected in a terminal plasma acid-soluble phosphate specific activity of 301 ± 74 compared to 91.9 ± 6.8 in the controls. However, the fact that the intima showed no such difference might be explained on two grounds: (*a*) the small number of animals in this experiment and the presence of wide fluctuations, and (*b*) the 20 per cent greater phospholipid content of the lesions in the eviscerated-nephrectomized group.

TABLE 5. PHOSPHOLIPID AND CHOLESTEROL CONCENTRATIONS IN TISSUES OF HYPERLIPEMIC AND NORMOLIPEMIC RABBITS—EXPERIMENT 1

	Phospholipids		Cholesterol	
	Hyperlipemic	Normolipemic	Hyperlipemic	Normolipemic
Liver	33.0 ± 3.0	32.8 ± 3.0	58.5 ± 12.6	5.61 ± 1.18
Plasma	8.32 ± 0.14	1.25 ± 0.09	21.4 ± 2.5	1.23 ± 0.32
Intima	25.2 ± 3.8	21.4 ± 2.6	81.0 ± 13.3	59.6 ± 5.0
Residual Aorta	9.90 ± 0.68	6.38 ± 0.35	20.5 ± 2.1	11.4 ± 1.3

Concentrations are expressed as mg./g. fresh tissue or /cc. plasma. Mean values of 4 animals ± standard errors.

creased to almost normal values but the atheromas persisted. These animals were compared to another group of four animals who were simultaneously maintained on rabbit chow for 2 months and subsequently on chow plus cholesterol for 3 months. Table 5 presents the phospholipid and cholesterol content of liver, plasma, aortic intima, and residual aorta (media and adventitia). Strictly speaking, a comparison of the extent of the lesions in the two groups might be misleading since the period of hypercholesterolemia in the normolipemic group was longer than in the hyperlipemic group. This is due to the fact that high serum cholesterol values persist after removal of the dietary cholesterol. To control this factor we maintained a second group of six animals on a high cholesterol diet for 3 months followed by a 2-month period on Purina chow (normolipemic group as above), six rabbits on Purina chow for 1 month, followed by 4 months on 1 per cent cholesterol diet (hyperlipemic group), and six animals on Purina chow for the entire 5 months. The course of serum cholesterol levels of these three groups is presented in Figure 2. In agreement with observations of McMillan *et al.* (11) and Friedman and Byers (12), we found that serum cholesterol levels decreased gradually over a period of 2 months after cessation of cholesterol feeding, and were almost down to normal at the end of 2 months. Although the hyperlipemic group continued on a high cholesterol diet, it also showed an appreciable decrease of serum cholesterol levels after the first month but the levels plateaued at around 1000 mg. per 100 ml. A similar phenomenon has been reported previously by several workers (8, 13) after 3 months of cholesterol feeding. Table 6 lists the concentrations of phospholipids and cholesterol of liver, plasma, aortic intima, and residual aorta, as in Table 5. Here again the liver cholesterol concentrations had decreased markedly in the normolipemic group although they did not quite reach the values of normal controls. It is interesting that the liver weights of the normolipemic as well as the hyperlipemic animals were about twice those of the control animals, although body weights were not significantly different. Similarly, the weights of the thoracic aortas in all cholesterol-fed rabbits exceeded those of the controls. In the hyperlipemic group the mean aortic weight was 1.06 with a standard error of 0.11 g. (intimal weight 0.46 ± 0.06), in the normolipemic animals the weight averaged 1.29± 0.12 g. (intimal weight 0.62 ± 0.05), and in the controls only 0.52 ± 0.04 g. Thus the concentrations of cholesterol and phospholipids in the atherosclerotic arteries do not give a true index of the degree of accumulation of lipids in these organs. The residual aorta (media and adventitia) also showed an increase in cholesterol and phospho-

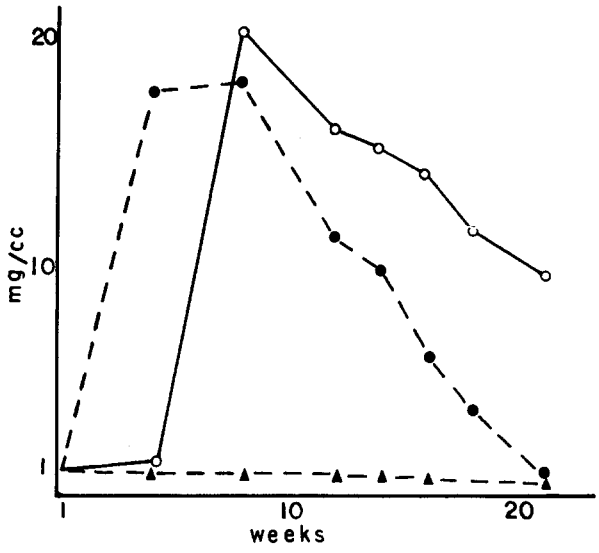

FIG. 2. Plasma cholesterol concentrations in control (--▲--), normolipemic (--●--) and hyperlipemic (—○—) rabbits at different intervals. Each point represents the mean of 5 or 6 animals. (Experiment 2, see text.)

TABLE 6. PHOSPHOLIPID AND CHOLESTEROL CONCENTRATIONS IN TISSUES OF HYPERLIPEMIC, NORMOLIPEMIC, AND CONTROL RABBITS—EXPERIMENT 2

	Phospholipids			Cholesterol		
No. of Animals	Control 5	Hyperlipemic 6	Normolipemic 5	Control 5	Hyperlipemic 6	Normolipemic 5
Liver	31.0 ± 1.8	27.0 ± 1.0	25.0 ± 1.0	2.19 ± 0.12	30.2 ± 4.27	8.20 ± 1.96
Plasma	0.728 ± 0.117	4.02 ± 0.71	1.18 ± 0.156	0.362 ± 0.053	7.91 ± 1.85	0.606 ± 0.330
Intima		27.5 ± 8.4	25.0 ± 3.8		64.3 ± 8.7	45.6 ± 9.5
Residual Aorta	5.12 ± 0.26	8.22 ± 0.97	8.42 ± 2.34	1.96 ± 0.47	11.6 ± 2.0	6.90 ± 1.80

Concentrations are expressed as mg./g. fresh tissue or /cc. plasma. Mean values ± standard errors.

lipid concentration, but it is difficult to tell whether this increase is the result of aortic tissue lipid or of lipid adhering to the adventitia. When one compares the total aortic cholesterol or phospholipid of the normolipemic groups in both experiments with that of the hyperlipemic groups, it is evident that no appreciable regression of the lesions had taken place as far as their lipid content is concerned.

In both experiments all animals received 0.5 mc. P^{32}-phosphate intravenously and 6 hours later were sacrificed for chemical and radioactive analyses. As there was no detectable difference in the data obtained from experiments 1 and 2, the radioactive analyses are presented in combined form in Table 7. As discussed previously in this paper, the specific activity of aortic phospholipids does not differ greatly in animals with severe lesions and those with few or no lesions. Therefore to get a better index of the rate of synthesis of phospholipids per aorta, the incorporations of injected P^{32} per whole thoracic aorta in the control, hyperlipemic, and normolipemic animals are compared. As before, we found that the incorporation of P^{32} in hypercholesterolemic animals is about six times greater than in normal controls. It is interesting, however, in this experiment that in those animals in which the blood lipids were allowed to drop to nearly normal values, the rate of incorporation of radioactive phosphate into phospholipids of the atheromatous aorta stayed at a level five times above normal and was not significantly different from the rate observed in the hyperlipemic animals. Apparently the rate at which P^{32} is incorporated into aortic phospholipids does not depend on the extent of serum lipid elevation.

DISCUSSION

From the above experiments no evidence could be obtained to suggest that deposition of plasma phospholipids is responsible for the incorporation of P^{32} into the phospholipids of arterial plaques. Previous studies with P^{32}-labeled lipoprotein also indicated that most of the radioactive phospholipid in the lesion was de-

TABLE 7. RADIOACTIVE DATA FROM HYPERLIPEMIC-NORMOLIPEMIC RABBITS (EXPERIMENTS 1 AND 2)

	Number of Animals	Specific Activity			Per Cent Injected Dose per Total Aorta × 10^3	
		Plasma	Aortic Intima	Residual Aorta	Aortic Intima	Residual Aorta
Control	5	5.10 ± 0.85		4.45 ± 0.69 *		0.461 ± 0.059 *
Hyperlipemic	10	2.19 ± 0.33	6.00 ± 0.75	6.29 ± 1.21	2.92 ± 0.71	1.09 ± 0.12
Normolipemic	9	3.65 ± 0.43	4.48 ± 0.50	4.30 ± 0.81	2.45 ± 0.28	0.528 ± 0.077

* Whole aortic wall, since separation of intima in control animals proved impractical. Phospholipid specific activity is expressed as per cent injected P^{32}/g. phospholipid P. Mean ± standard error.

rived from synthesis *in situ* (1). Although this evidence strongly suggests that all the excess phospholipid in the atheroma is derived from local synthesis, such a conclusion cannot be asserted with absolute certainty. A rough calculation of the rate of accumulation of arterial phospholipid during the period of cholesterol feeding shows that deposition of as little as 5 μg. of phospholipid P per hour could account for the phospholipid in the plaque. This rate is well below the sensitivity of our tracer measurements. Furthermore, our studies pertain only to the phosphorus portion of the phospholipid molecule. It is possible that phospholipids arrive at the intima by transport from plasma and that the phosphorus portion exchanges rapidly with the phosphorus pool of the intima. Studies with labeled fatty acids might yield additional evidence on this point. Should one therefore wish to preserve the idea that plaque phospholipids are derived from the deposition of plasma lipid or lipoprotein, our experiments could not strictly reject such a hypothesis. On the other hand, it would appear that the deposition hypothesis, for phospholipids at least, is based in part on analogies with the behavior of cholesterol. Even for the latter substance direct proof of lipid deposition is unavailable to date, although suggestive evidence has been presented. In the absence, then, of good reasons for assuming phospholipid deposition and with the availability of evidence for accelerated production of phospholipids in the lipid-laden intima, it would appear that the concept that all excess phospholipid in the atheroma is derived from synthesis *in situ* is a reasonable working hypothesis.

The implications of such a hypothesis are manifold. One should like to know whether the phospholipids in the atheroma are part of the initial injury to the artery or whether they might serve to protect the arterial intima from cholesterol accumulation. The well-known surface active properties of phospholipids would appear to lend credence to the speculation that the accelerated turnover of intimal phospholipids aids in the removal of cholesterol by solubilization of the latter from the arterial wall into the plasma. Such action would appear to be specific to the atheroma since in other tissues, including the cholesterol-rich liver, no increase in phospholipid P^{31} and P^{32} concentration is observed. This should not be too surprising, however, in view of the fact that the liver can dispose of cholesterol by excretion of the sterol itself or its derivative, bile acid. Moreover, the solubilization of liver cholesterol by excess liver phospholipid synthesis would increase the serum cholesterol level, which would presumably not enhance the survival of the animal. The aorta, on the other hand, does not have an excretory mechanism like the liver, and unless cholesterol can be oxidized by arteries (about which no data are available), solubilization of cholesterol might be the only mechanism whereby the artery could rid itself of excess cholesterol. To what extent these findings and speculations are applicable to human arterial disease is not known at the present time and must await further metabolic work on human arterial tissues.

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