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STUDIES ON THE PATHOGENESIS OF VASCULAR DISEASE

The Effect of Intravenously Injected Human Plasma and of Lipid-Rich Human Plasma Globulins on Inflammatory Lesions of the Coronary Arteries of Dogs**

Investigations of Gofman and his associates[4] have directed attention to the plasma lipoproteins in persons with clinical manifestations of arteriosclerosis. Recently, lipoproteins with flotational characteristics similar to those in serum have been extracted from diseased human aortas.[5] Some direct evidence for the participation of these substances in the genesis of experimental arterial changes in animals has also been reported: Bragdon[1,2,3] injected rats intravenously with material derived from plasma lipoproteins of hypercholesterolemic rabbits and obtained scattered fatty arterial lesions. The experiment in rats was repeated, substituting human lipoprotein fractions, with a similar result. From this laboratory preliminary report has been made of experiments indicating that lipids localize selectively in areas of concomitant arterial injury in dogs following the intravenous injection of human plasma or of lipoprotein fractions.[15,16,17] The report which follows describes these studies in further detail.

The general plan of the investigation has been to observe the effect of intravenously injected human plasma or of lipid-rich human plasma globulins on the basic morphological sequences of lesions of dogs' coronary arteries injured by the administration of allylamine.

MATERIALS AND METHODS

Adult mongrel dogs maintained on a low-fat, standard laboratory diet were utilized. The technique for the production of lesions of the coronary arteries of dogs by allylamine has been described previously.[12] In brief, 15-20 mg./kg. of neutralized allylamine in a 1 per cent aqueous solution were injected into dogs intravenously on each of the first three days of the experiment, or on days 1, 3, and 4, or on 1, 2, and 4,

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depending on the tolerance of the animal. With this regimen, 80-90 per cent of the animals exhibited multiple lesions of their coronary arteries when examined from three days to two weeks after the beginning of the experiments. In any one animal the lesions varied in intensity, ranging from small areas of medial edema and necrosis to necrotizing panarteritis with intimal proliferation and partial or complete obliteration of the involved segment. Control allylamine lesions contained at most traces of stainable fat and progressed to scar.

Clear human "plasma" for injection was prepared by centrifuging recently outdated, but nonhemolyzed ACD blood (whole blood approximately 440 ml., Anticoagulant Acid Citrate Dextrose Solution B., U.S.P. 110 ml.) at low speed and by subsequently separating the plasma-preservative mixture. The latter was now centrifuged at 20,000 r.p.m. for one hour to remove large fatty particles (chylomicrons) and any other insoluble material. In a group of eight dogs, following the allylamine injections, the clear plasma-preservative mixture was given over periods of from 30 minutes to seven days in total amounts varying from 300 to 5,125 ml. (See Table 8). Such acute augmentation of blood volume may in itself lead to damage of dogs' coronary arteries, as has been demonstrated.[18] However, the combined vascular-damaging effects of allylamine and of this procedure were not appreciably different from those of allylamine alone.

Lactescent human plasma was obtained by centrifuging chylomicron-rich bank blood at low speed and by separating the plasma with precautions for sterility. Lactescent plasma was administered to a series of five allylamine-injected animals in varying large amounts as set forth in Table 9.

The starting material for the preparation of lipid-rich protein fractions of human plasma was also sterile ACD bank plasma from recently outdated, nonhemolyzed bank blood. After centrifugation at 20,000 r.p.m. for an hour to remove chylomicron-like particles, the plasma was treated with an aqueous solution of polyethylene glycols (carbowaxes*) which effected, at room temperature and at a pH of 6.7-6.9, the precipitation of an undenatured lipid-rich protein complex. The precipitant polyethylene glycol solution was prepared as follows: To nine parts of melted carbowax 4,000, one part of melted carbowax 1,500 was added. An equal volume of sterile distilled water was added to the melted glycols, a 50 per cent aqueous solution (pH 5.2) of the mixture resulting. This preparation was used in the precipitations to be described. (The proportions of carbowax apparently may be varied as certain other mixtures have also proven effective. However, a survey of optimal polyethylene glycol preparations for the precipitation of the protein fractions of plasma was beyond the scope of the present investigation.)

For precipitation of the lipid-rich plasma protein fraction utilized in these experiments, 25 ml. of the 50 per cent aqueous carbowax solution were slowly added with stirring to each 100 ml. of plasma. After complete precipitation, the yellow-orange precipitate was separated by centrifugation. From four samples of ACD human plasma, the average recoveries of lipids and of proteins in the fraction were as shown in Table 1.

The crude, lipid-rich plasma protein precipitate was readily soluble in physiological saline and upon the addition of water. This precipitate, dissolved in 0.9 per cent saline,

* Carbowaxes are products of the Union Carbide and Carbon Corporation, New York, New York.

was used in the first few animals, but thereafter further purification was carried out. No differences in the physiological or pathological effects of the crude or more purified materials were noted. In preparation for the majority of the experiments, the first carbowax precipitate was dissolved in a volume of distilled water equal to the original plasma volume and was then allowed to stand overnight at 4° C. A lipid-poor, gray-white precipitate separated. This was removed by centrifugation, and the clear, light-yellow supernatant was reprecipitated with 50 per cent aqueous carbowax as before. The resultant precipitate was homogenous in texture and light-yellow in color. Its solubility was such that five- to sixfold concentrates in saline were possible. As a

TABLE 1. LIPIDS AND PROTEIN REMOVED FROM ACD PLASMA BY PRECIPITATION WITH POLYETHYLENE GLYCOLS (AVERAGE OF 4 SAMPLES)

	Per cent of total
Total cholesterol	82.8
Free cholesterol	100.0
Fatty acids	58.1
Lipid phosphorus	51.1
Protein	30.7

Actual Values Obtained in a Sample Analysis

Material	*Total cholesterol mg.%*	*Free cholesterol mg.%*	*Fatty acids mEq./l.*	*Lipid phosphorus mg.%*	*Total protein gm.%*
Control plasma	174.3	31.5	9.7	7.1	5.2
Carbowax fraction	138.7	36.8	6.3	4.1	1.6
Duplicate fraction	138.7	33.8	6.3	3.8	1.2
Triplicate fraction	132.5	33.8	6.4	3.7	.95

usual procedure for injection, the precipitate from 1,800 ml. of blood-bank plasma was redissolved in 300 ml. of 0.9 per cent saline. On standing, a slight lipid-poor precipitate separated. After removal of this material, the clear, sterile concentrated protein solution (pH 6.5-6.7) was utilized for injection. The pertinent components of a typical concentrate are given in Table 2.

The majority of the plasma protein solutions utilized contained residual amounts of the precipitant glycols. However, the injection of this fraction into dogs in concentrated form did not lead to the known renal or hepatic lesions of polyethylene glycol poisoning. In control experiments it was found that the amount of injected pure glycol associated with the appearance of such lesions in dogs greatly exceeded that present in the lipid-rich protein fractions.

In any case, experience was gained with a method for the removal of residual polyethylene glycols from the lipid-rich plasma globulin fractions. The injection of this carbowax-free material resulted in the same vascular lesions as the less purified globulin fractions (See Table 10, Dog No. 1982.). Glycols were removed from the

fractions by the following procedure: Initial precipitates from blood-bank plasma were dissolved in distilled water and were allowed to stand overnight at 4° C. A gray-white, lipid-poor precipitate was separated by low-speed centrifugation. The supernatant was then treated with the glycol mixture. The resultant precipitate was washed repeatedly with distilled water in which, as a mixture of globulins, it was only sparingly soluble. Three to five washings sufficed to remove carbowaxes from the fraction. Completeness of removal was determined by the sensitive colorimetric method of Shaffer and Critchfield.[11] Losses of lipid-containing proteins in the supernatants, particularly in the first washing, were considerable. This appeared to be a matter of ionic strength, since the globulin could be partially recovered by dilution or by dialysis of the supernatant.

TABLE 2. LIPID AND PROTEIN CONTENT OF A PLASMA PROTEIN CONCENTRATE USED FOR INJECTION

Total cholesterol	725.0 mg.%
Free cholesterol	224.9 mg.%
Fatty acids	40.0 mEq./l.
Lipid phosphorus	23.4 mg.%
Total protein (globulin)	7.0 gm.%

TABLE 3. RECOVERY OF LIPIDS AND OF PROTEIN IN CARBOWAX-FREE GLOBULIN FRACTION OF ACD HUMAN PLASMA (AVERAGE OF FOUR SAMPLES)

	Per cent of total
Total cholesterol	58.6
Free cholesterol	66.7
Fatty acids	49.3
Lipid phosphorus	55.2
Protein (globulin)	7.5

Without reworking of the supernatants, recoveries of plasma lipids and of proteins in the purified material from bank plasma were as shown in Table 3.

Not only was the residual carbowax removed, but there was also elimination of much lipid-poor protein from the fraction. Large variation in the amounts of lipids recovered occurred among samples. This was believed to be associated at least in part with differences in the age and in the composition of the original plasma-preservative mixtures.

The procedure described for the preparation of a carbowax-free protein fraction of blood-bank plasma was also effective with fresh human serum or with fresh dog serum. However, with these materials the precipitations often occurred more slowly, and high speed centrifugation was sometimes necessary to effect the separations. Of considerable interest was the observation that the glycol-precipitable fraction of fresh

dog serum contained, in contrast to that from human serum, only a small fraction of the total serum lipids.

The washed, carbowax-free, lipid-rich protein fraction from human plasma was soft, sticky, and a homogeneous bright yellow in color. In saline, at pH 6.5-6.7 and on standing, a further fine nonlipid-containing protein precipitate separated. This did not occur in phosphate buffer at pH 7.4. At room temperature, ether did not extract appreciable quantities of lipid from saline solutions of the fraction. However, when precipitated

TABLE 4. LIPID AND PROTEIN CONTENT OF A CARBOWAX-FREE GLOBULIN CONCENTRATE FROM HUMAN PLASMA

Total cholesterol	610.0 mg.%
Free cholesterol	180.0 mg.%
Fatty acids	36.3 mEq./l.
Lipid phosphorus	17.8 mg.%
Protein (globulin)	2.2 gm.%

TABLE 5. THE EFFECT OF DAILY INFUSIONS OF CLEAR ACD HUMAN PLASMA* ON THE PLASMA LIPIDS OF ALLYLAMINE-TREATED DOG NO. 1387—WEIGHT 10.0 KG.

Day	*Plasma injected, ml.*	*Total cholesterol mg.%*	*Free cholesterol mg.%*	*Fatty acids mEq./l.*	*Lipid phosphorus mg.%*
Control	...	117.6	29.4	8.3	8.5
1	600	166.0	48.5	13.2	9.0
2	725	257.3	75.5	18.0	9.3
3	700	282.0	89.0	23.0	12.6
4	350	297.0	90.0	18.6	16.6
5	600	323.7	102.5	17.5	16.4
6	350	315.0	92.5	13.0	14.5
7	375	323.7	89.5	13.0	14.0
* Pooled clear ACD human plasma used in the above experiment:		156.8	46.9	16.0	12.2

from solution by carbowax, lipids were readily extracted from the protein by ether. Thin frozen sections of the precipitated protein fixed in formalin stained diffusely with sudan dyes. The fresh precipitate dried on a slide gave an intense Schultz reaction for steroids.

Chemical and electrophoretic* analyses of the carbowax-free fraction revealed it to be a mixture of alpha, beta, and gamma globulins. Paper electrophoresis** demon-

* Through the kindness of Dr. J. Haskell Milstone.
** Through the courtesy of Drs. K. Kirkeby and Gerald Klatskin.

strated the contained lipids chiefly in the beta globulin zone, with appreciable quantities migrating with the alpha globulins.

The lipid and protein content of the carbowax-free globulin concentrate given dog No. 1982 appears in Table 4.

Dogs with injuries of their coronary arteries associated with allylamine injections were given repeated intravenous infusions of clear saline solutions of concentrated lipid-rich plasma globulins. The observed effects on the injured segments of the blood vessels were the same with either the crude or more purified fractions. Depending on the condition of the animals or the objective of the particular experiment, there was

TABLE 6. THE EFFECT OF DAILY INFUSIONS OF ACD LACTESCENT HUMAN PLASMA* ON THE PLASMA LIPIDS OF ALLYLAMINE-TREATED DOG NO. 1281. WEIGHT 10.9 KG.

Day	*Plasma injected, ml.*	*Total cholesterol mg.%*	*Free cholesterol mg.%*	*Fatty acids mEq./l.*	*Lipid phosphorus mg.%*
Control	...	154.0	42.5	12.8	14.5
1	850	315.0	92.5	19.0	17.5
2	600	307.0	85.0	16.0	18.4
3	500	218.0	61.5	13.0	20.5
4	550	191.0	56.0	13.0	20.5
5	200	218.0	70.0	12.0	20.0
6	300	188.0	65.0	12.6	14.4

* Pooled lactescent ACD human plasma used in the above experiment:

	186.8	69.0	25.5	14.1

variation in the amounts of protein injected and in the schedules of injections. The results described in this section of the report are based on observations of 17 dogs receiving three daily injections of allylamine followed by 1 to 9 injections of concentrated lipid-rich plasma protein (See Table 10). The animals were sacrificed at varying times after the combined procedures, that is, from one hour after the first injection of globulin to two days after a course of 9 daily injections.

Histological study of the cardiovascular systems of all animals was carried out at the termination of the experiments. Frozen sections stained with sudan IV were prepared for the demonstration of lipids in tissues. A modified Schultz test was utilized for tissue steroids. Paraffin sections were stained with hematoxylin and eosin, Masson's trichrome stain, or Verhoeff's method for elastic tissue. Lipid determinations on the plasma and plasma fractions injected and on the recipient dogs' blood were made using a modified Schoenheimer-Sperry method for cholesterol,[10] a modified Youngburg procedure for lipid phosphorus,[6] and the procedure of Man and Gildea for fatty acids.[7] Nitrogen determinations for estimation of serum proteins were by the usual micro-Kjeldahl procedure.

EXPERIMENTAL RESULTS

The effect of large intravenous injections of human plasma or of lipid-rich plasma protein fractions in dogs.

Dogs tolerated injections of moderate amounts of human plasma well. Massive infusions, above 100 ml./kg., and sometimes less, led to circulatory overload. This limited the amount of human plasma that could be injected into the animals. It was difficult to maintain the dogs' blood lipids, other than lipid phosphorus, above maximal levels[9] for normal humans. A princi-

TABLE 7A. THE EFFECT OF DAILY INFUSIONS OF LIPID-RICH HUMAN PLASMA GLOBULINS* ON THE PLASMA LIPIDS OF ALLYLAMINE-TREATED DOG NO. 1501. WEIGHT 6.8 KG.

Day	*Globulin injected, ml.*	*Total cholesterol mg.%*	*Free cholesterol mg.%*	*Fatty acids mEq./l.*	*Lipid phosphorus mg.%*
Control	...	107.9	41.3	14.7	13.3
1	300	214.2	84.0	17.0	27.9
2	300	344.4	109.2	21.0	28.5
3	300	294.0	109.2	19.3	27.0
4	300	346.1	116.8	19.3	26.0
5	300	416.7	116.8	27.0	28.0
6	300	416.7	122.6	25.3	26.0
7	300	427.5	151.6	26.6	27.0

* For lipid and protein content of this solution, see Table 2.

pal reason for this limitation was dilution of the injected plasma with the dogs' own blood. It should be emphasized that the plasma of dogs given clear human plasma or clear solutions of human plasma globulin did not become lactescent during the period of the experiment. The effect of intravenous injections of clear or of lactescent human plasma on the blood lipids of dogs is illustrated in Tables 5 and 6.

Exchange transfusions were not attempted. Anaphylactic phenomena in the dogs given human plasma or plasma fractions were only occasionally severe, even with repeated injections. Circulatory overload with massive injections of plasma was the only major technical problem. This was circumvented in other animals by injecting concentrated lipid-rich plasma proteins as described. Large quantities of human plasma globulin were given in this way and the recipients' blood lipids became markedly elevated. Values for the plasma lipids of dogs receiving repeated injections of lipid-rich human globulins are given in Tables 7a and 7b.

Vascular lesions in dogs following allylamine injections and repeated infusions of human plasma.

Eight dogs were given allylamine and clear human plasma, and five animals received allylamine and lactescent human plasma. Protocols of these experiments are summarized in Tables 8 and 9. Necrotizing lesions of the coronary arteries occurred in varying degrees of severity in all but one of these animals. Lipids accumulated rapidly at the sites of arterial injury both in the group receiving clear plasma and in the group receiving lactescent

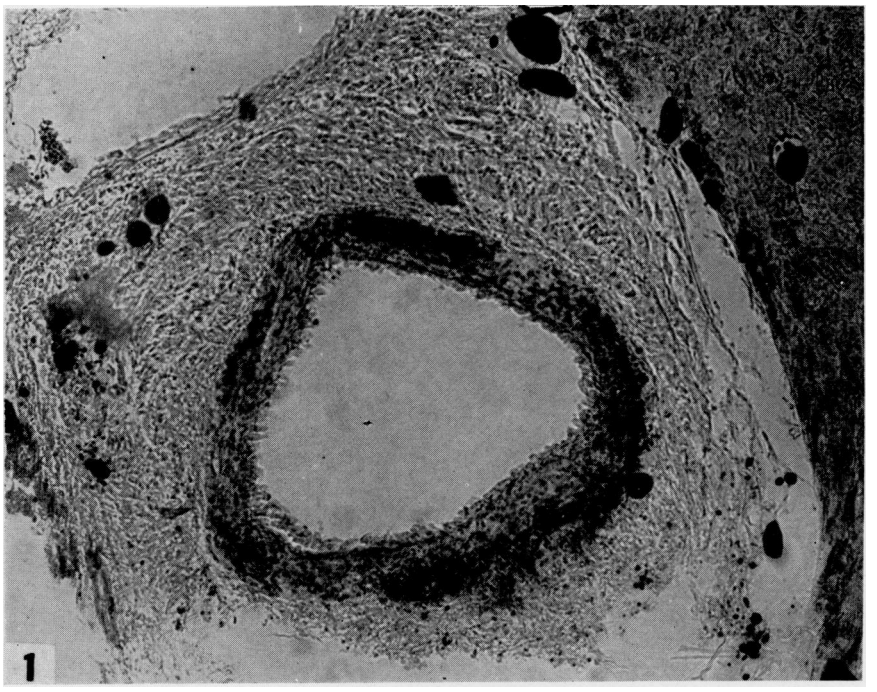
TABLE 7B. VALUES FOR PLASMA LIPIDS AND SERUM PROTEINS OF ALLYLAMINE-TREATED DOG NO. 1982, WEIGHT 6.0 KG., RECEIVING CARBOWAX-FREE HUMAN PLASMA GLOBULIN*

Day	*Globulin injected, ml.*	*Total cholesterol mg.%*	*Free cholesterol mg.%*	*Fatty acids mEq./l.*	*Lipid phosphorus mg.%*	*Serum protein Gm. %*
Control	...	139.5	31.5	10.0	10.7	6.06
1	250	....	....	...	...	
2	300	....	....	...	...	
3	250	....	....	...	...	
4	...	666.0	247.8	26.0	25.0	6.19
5	...	....	....	...	...	
6	...	....	....	...	...	
7	...	140.0	46.2	10.0	9.8	5.72

* For lipid and protein content of this preparation, see Table 4.

plasma. Control arterial lesions, associated with injections of allylamine alone, did not stain with sudan dyes. Lipids in the lesions of the experimental animals appeared first as a diffuse medial sudanophilia, detectable within an hour after the first plasma injection. With the passage of time, the sudan-positive material became concentrated into intensely staining globules (Figs. 1 and 2). With lactescent plasma administration, the final accumulation of lipids in the areas of injury was only slightly greater than in the arteries of animals receiving clear human plasma. In both groups, as the lesions became older (5-10 days), phagocytosis of the accumulated lipid droplets was apparent (Fig. 3). In one animal (Dog No. 1281 of the lactescent plasma series) this process became prominent enough to suggest xanthomatous transformation of the intimal proliferation (Fig. 4).

In several animals receiving allylamine and repeated injections of clear or lactescent human plasma, a light diffuse sudanophilia of the inner third of



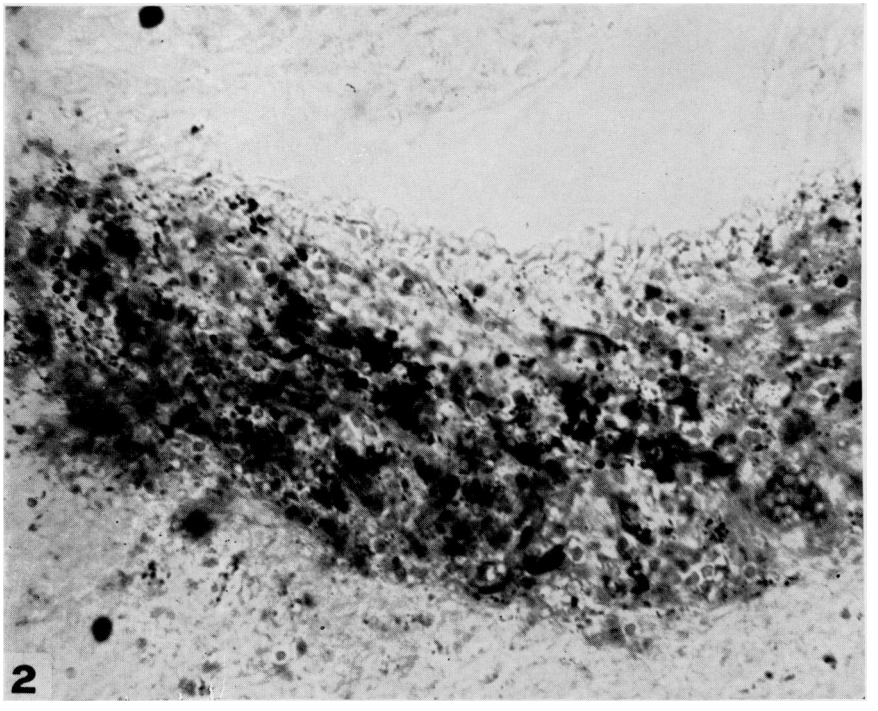
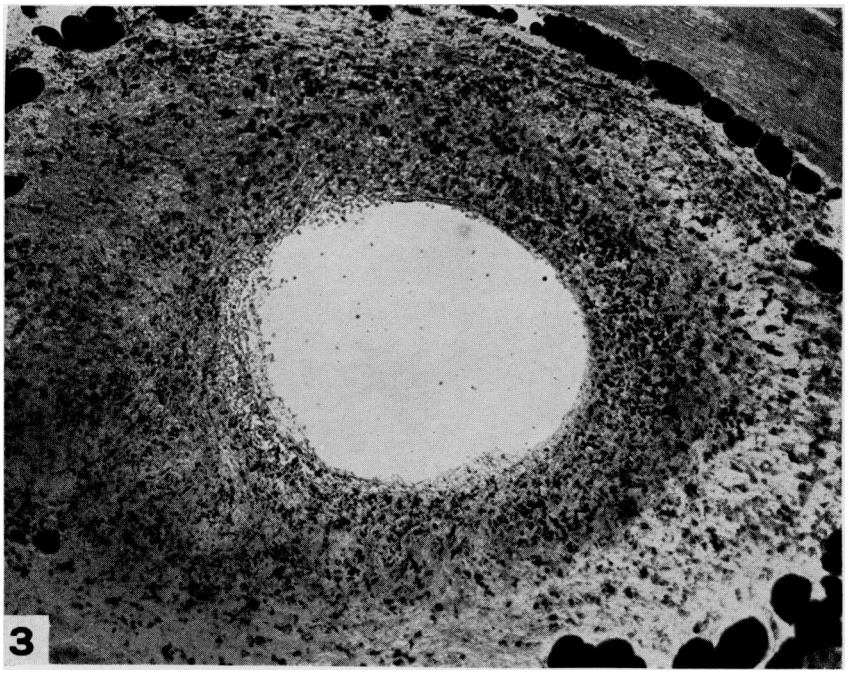


FIG. 1. Coronary artery, dog 1433. Allylamine and lactescent human plasma, three days. Sudan IV stain. The dark masses in the media represent lipids in diffuse and droplet form.

FIG. 2. High power view of segment of a coronary artery of dog 1254. Allylamine and clear human plasma, five days. Sudan IV stain. The dark material in the media is lipid in diffuse and droplet form. Many droplets are within phagocytes.



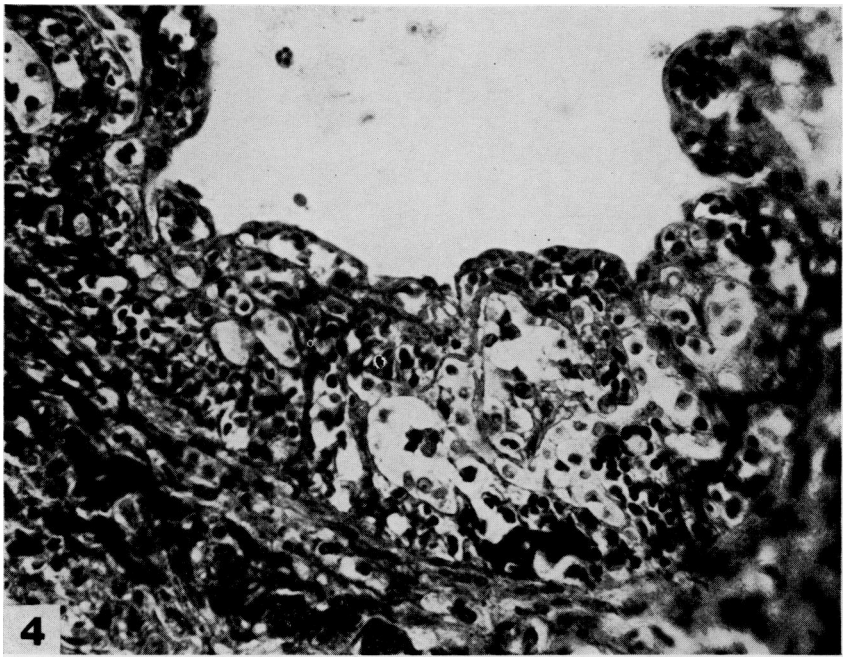
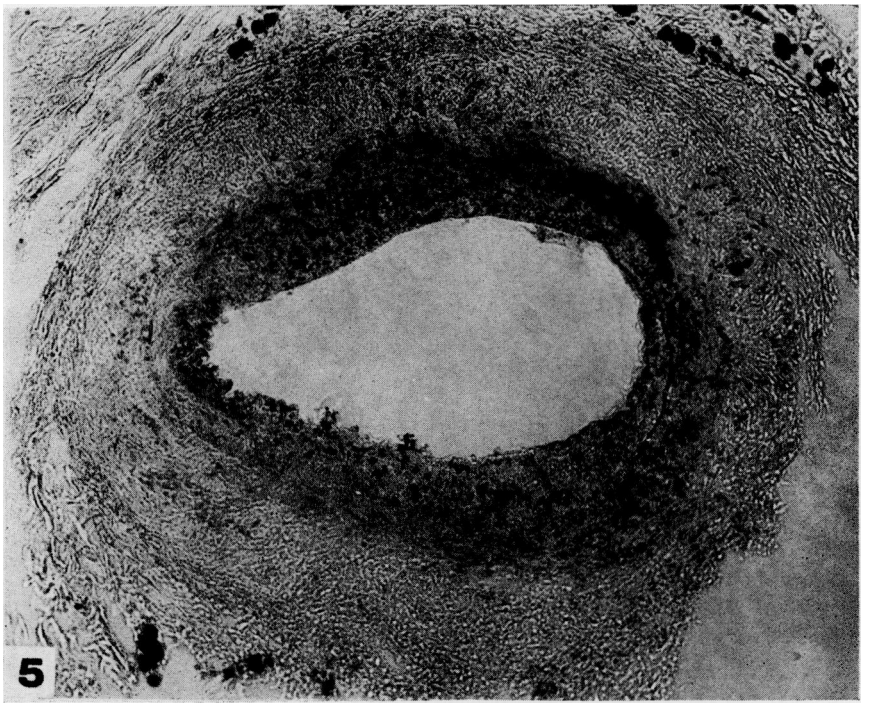


FIG. 3. Coronary artery, dog 1387. Allylamine and clear human plasma, ten days. Sudan IV stain. The dark-staining material in the media and adventitia is phagocytized lipid. Large dark fat cells surround the area.

FIG. 4. High power view of the proliferated intima of a coronary artery of dog 1281. Allylamine and lactescent human plasma, ten days. H & E stain. Accumulations of large, pale lipophages are present in the subendothelial tissue. Red blood cells, lymphocytes and dark masses of "fibrinoid" material are also seen.



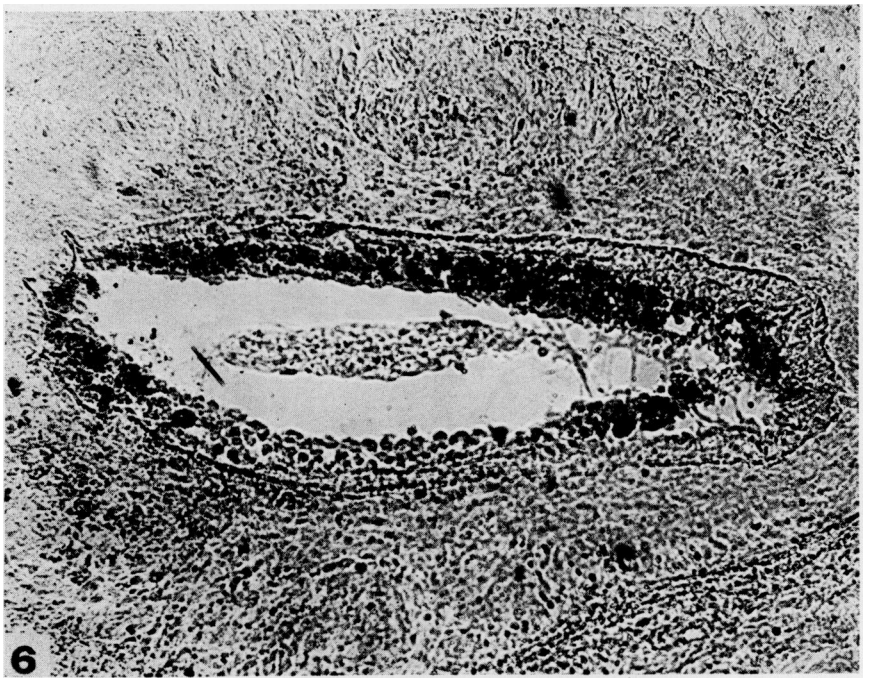
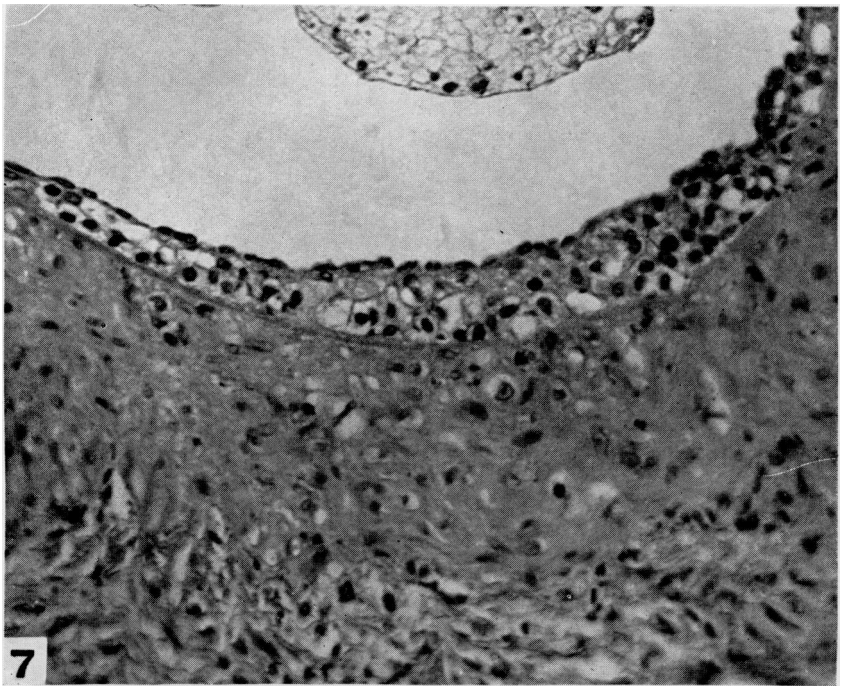


FIG. 5. Coronary artery, dog 1982. Allylamine and lipid-rich human plasma globulin, seven days. Sudan IV stain. The dark areas represent diffuse and phagocytized lipid in the proliferated intima and in the media.

FIG. 6. Coronary artery, dog 1450. Allylamine and lipid-rich human plasma globulin, nine days. Sudan IV stain. Fatty foam-cellular intimal lesion.



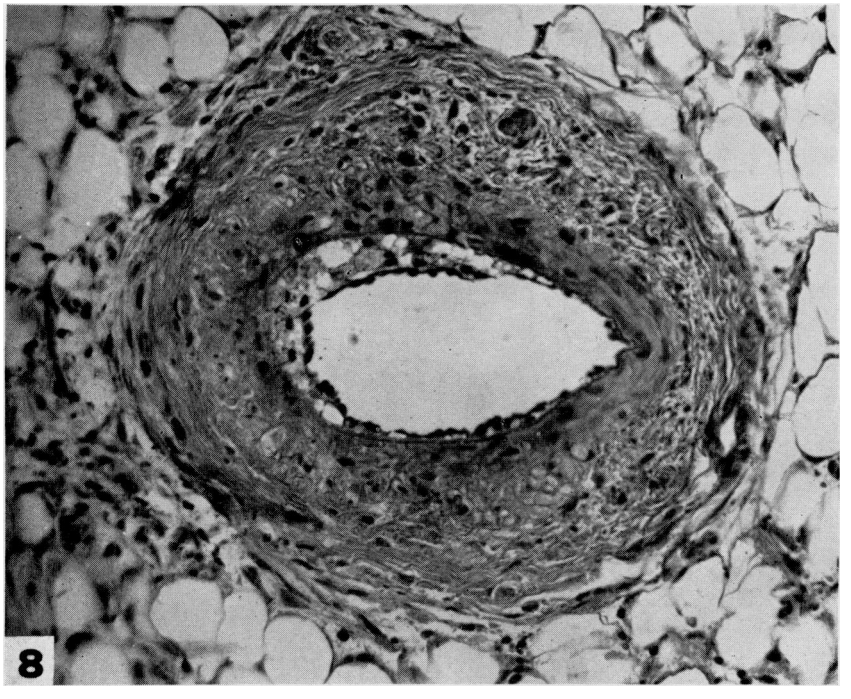
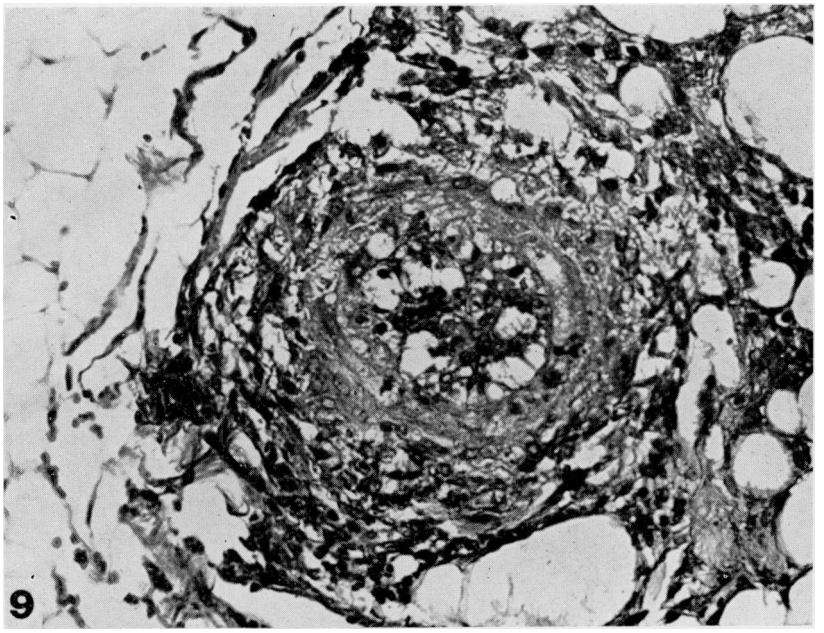


FIG. 7. Segment of coronary artery, dog 1455. Allylamine and lipid-rich human plasma globulin, eight days. H & E stain. Fatty foam-cellular intimal plaque.

FIG. 8. Coronary artery, dog 1457. Allylamine and lipid-rich human plasma globulin, nine days. H & E stain. Crescentic foam-cellular intimal proliferation.



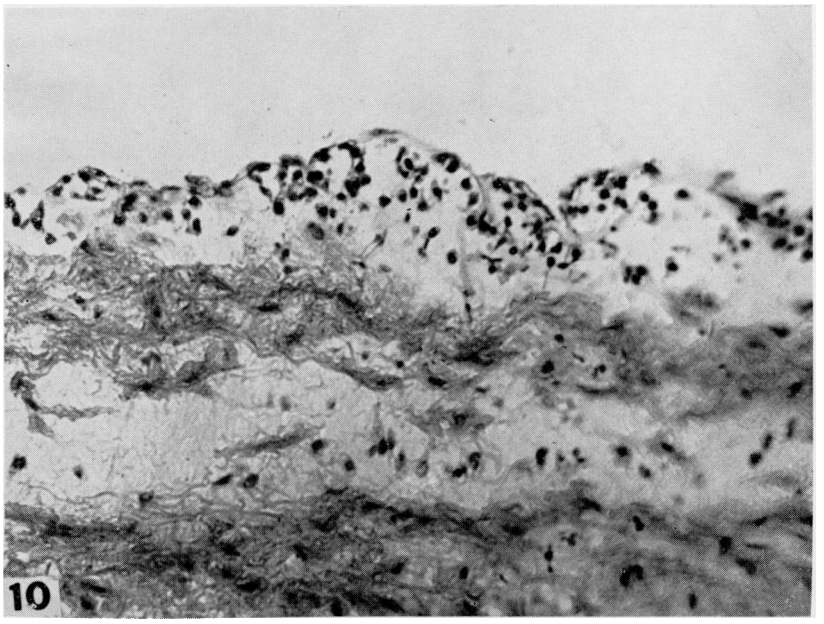


FIG. 9. Small epicardial coronary artery, dog 1982. Allylamine and lipid-rich human plasma globulin, seven days. H & E stain. A fatty granuloma has occluded the lumen.

FIG. 10. Aorta, dog 1982, intimal surface above. Allylamine and lipid-rich human plasma globulin, seven days. H & E stain. The endothelium is raised and there are mononuclear cells and phagocytes in the subendothelial space and in the edematous media.

the aorta, including the intima and inner media, developed. This lesion occasionally exhibited lipid droplet formation and phagocytosis of fatty particles.

TABLE 8. DOGS RECEIVING ALLYLAMINE (15-20 MG./KG., 3 DOSES) AND CLEAR HUMAN PLASMA

Dog No.	*Wt. (kg.)*	*Plasma injections*	*Total plasma injected, ml.*	*Duration of experiment after first plasma injection*		*Vascular lesions*
W 1214	9.6	1	300	30 minutes	S	Medial necrosis of coronary arteries. The injured areas stain diffusely and intensely with sudan IV.
W 1224	8.4	1	500	30 minutes	D	"
W 1232	10.6	1	600	1 hour	S	"
W 1254	12.2	3 in 5 days	700	5 days	S	Medial necrosis of coronary arteries. Lipid in these lesions is in diffuse and droplet form (Fig. 2). Diffuse sudanophilia of inner third of aorta.
W 1255	7.0	5 in 5 days	1150	8 days	S	No allylamine lesions, no lipid deposits.
W 1308	8.2	7 in 7 days	1850	12 days	S	Plasma in lumens of vessels sudanophilic. Much lipid at sites of injury in coronary arteries, in diffuse and droplet form. Scattered lipophages. Lipid in inner third of aorta.
W 1387	10.0	11 in 7 days	3700	10 days	S	" (Fig. 3).
W 1323	10.6	12 in 6 days	5125	12 days	S	"

D = Died, S = Sacrificed.

Lesions of the arteries of dogs after a course of allylamine and a single intravenous injection of concentrated, lipid-rich, human plasma globulin.

Within an hour, the sites of acute arterial injury were diffusely sudanophilic (Table 10). Wherever plasma was observed microscopically in the lumens of vessels, it stained diffusely with sudan IV, in a manner entirely analogous to the staining of the injured mural arterial segments. The lipid staining of these sites was therefore believed to be associated with the entrance of lipid-rich plasma into the vessel walls in areas of increased

permeability. The hearts and blood vessels of dogs were also examined at 2, 6, 24 hours, and four and five days after a single injection of lipid-rich globulin. At these intervals, a progressively increasing number of sudano-

TABLE 9. DOGS RECEIVING ALLYLAMINE (15-20 MG./KG., 3 DOSES) AND LACTESCENT HUMAN PLASMA

Dog No.	*Wt. (kg.)*	*Plasma injections*	*Total plasma injected, ml.*	*Duration of experiment after first plasma injection*		*Vascular lesions*
W 1301	7.2	4 in 4 days	200	8 days	S	Medial necrosis and intimal proliferation of coronary arteries. Slight deposit of lipid in droplet form.
W 1433	8.0	2 in 2 days	900	3 days	S	Medial necrosis of coronary arteries. Deposits of lipid in droplet form and diffusely (Fig. 1).
W 1410	13.0	7 in 4 days	2600	8 days	S	Medial necrosis and intimal proliferation of coronary arteries. Deposits of lipid in droplet form. Scattered lipophages.
W 1281	10.9	11 in 6 days	3000	10 days	S	Medial necrosis, intimal proliferation of coronary arteries; deposits of lipid in droplet form. Many lipophages (Fig. 4).
W 1309	9.5	12 in 6 days	4150	11 days	S	Medial necrosis, intimal proliferation of coronary arteries. Diffuse lipid and lipid in droplet form. Scattered lipophage reaction.

S = Sacrificed.

philic droplets were present at the sites of arterial injury, in a background of diffuse sudanophilia. At the longer time intervals, lipophage activity had already begun.

Changes in the coronary arteries and aortas of dogs following a course of allylamine and repeated injections of lipid-rich human plasma globulin.

At the sites of coronary artery injury in dogs given combined courses of allylamine and of the lipid-rich globulins of human plasma, fatty foam-cellular intimal granulomata were found. Details are given in Table 10. As the animals were sacrificed at varying time intervals, the development of these lesions could be observed. Following the accumulation of sudanophilic

TABLE 10. DOGS RECEIVING ALLYLAMINE (15-20 MG./KG., 3 DOSES) AND LIPID-RICH HUMAN PLASMA GLOBULIN

Dog No.	*Wt. (kg.)*	*Globulin injections*	*Total globulin injected, ml.*	*Duration of experiment after first globulin injection*		*Vascular lesions*
1500	7.0	1	500	1 hour	S	Medial necrosis of coronary arteries; diffuse sudanophilia of injured sites and of plasma in vascular lesions.
1505	6.3	1	600	1 hour	D	"
1597	8.0	1	600	2 hours	S	"
1503	7.0	1	600	6 hours	D	Medial necrosis of coronary arteries; diffuse sudanophilia of injured sites and of plasma. Also beginning lipid droplet formation in areas of injury.
1474	9.5	1	600	24 hours	D	Same as 1503 above, but with many lipid droplets and less diffuse lipid at injured arterial sites. Scattered lipophages.
1438	6.8	1 (crude)	250	3 days	S	No allylamine lesions; no lipid deposits.
1513	8.6	1	600	4 days	S	Medial necrosis of coronary arteries; deposit of fine lipid droplets at sites of arterial injury, mostly extracellular. Scattered lipophages.
1446	7.0	2 (crude)	750	4 days	D	"
1490	9.1	2	800	4 days	S	"
1498	10.0	2	900	4 days	S	"
1514	10.0	1	600	5 days	S	Few medial lesions of coronary arteries with deposited granular lipid; scattered lipophages.
1455	10.0	3	1500	8 days	S	Many foamy sub-intimal and medial lesions of coronary arteries (Fig. 7).

D = Died, S = Sacrificed.

Dog No.	Wt. (kg.)	Globulin injections	Total globulin injected, ml.	Duration of experiment after first globulin injection		Vascular lesions
1501	6.8	7	2100	9 days	S	Many medial lesions of coronary arteries with droplets and irregular masses of lipid. Occasional intimal foam-cell proliferations.
1457	8.0	8	2300	9 days	S	Many foam-cell intimal lesions of coronary arteries where vessels are proliferating; also many fatty medial lesions (Fig. 8).
1450	10.0	7	2920	9 days	S	Medial lesions of several coronary arteries containing droplet lipid; foam-cell intimal lesions of auricular vessels, which have proliferated (Fig. 6).
1338	8.0	9 (crude)	3000	11 days	S	Intimal and medial lipid-containing lesions in coronary arteries. Foam-cell changes occur chiefly in the small left ventricular branches.
1982	6.0	3 (carbowax-free)	800	7 days	S	Massive xanthomatous intimal and medial lesions in coronary arteries. Lipid-containing subendothelial proliferations of aorta (Figs. 5, 9, 10).

S = Sacrificed.

droplets in the area of medial necrosis, lipophages appeared in the media and in the subendothelial space. The accumulation of and the increase in size of these cells led to xanthomatous transformation of the basic intimal allylamine lesion. Changes characterized by phagocytosis were well developed 7-9 days after the first globulin injection and 10-12 days after the beginning of the experiment (Figs. 5, 6, 7, 8, 9). The intimal fatty proliferations often deformed or partially occluded the lumen of the involved artery. The morphological similarity of many of these lesions to the fatty foam-cellular accumulations occurring in arteriosclerosis in man was striking. Uninjured segments of the vessels contained no lipids. In surveying the histological material, it became apparent that the greater the amount of globulin injected or the higher the level of the blood lipids obtained, the more frequent and further developed were the fatty arterial lesions.

After allylamine and repeated injections of plasma globulins, the connective tissue spaces of the inner third of the aortic wall stained diffusely with sudan IV. Occasionally there was evidence of lipid droplet formation, of the accumulation of lipophages, and of subendothelial connective tissue proliferation (Fig. 10).

DISCUSSION

The experiments described in this report indicate that following the injection of clear or of lactescent human plasma or of lipid-rich human plasma globulins into allylamine-treated dogs, there was an accumulation of sudanophilic materials selectively at sites of acute arterial inflammation. After injection of the globulin fractions, xanthomatous transformation of the basic arterial lesions occurred as well. The lipid-connective tissue reactions in the experimental lesions found morphological counterparts in the intimal fatty and foam-cellular changes of arteriosclerosis in man. No lipids were present in *uninjured* segments of the arteries of the experimental animals even in subjects in which values for the blood lipids were greatly elevated during the observation period. This finding stresses the dependence of the intramural lipid accumulations on prior arterial injury.

With injections of clear or of lactescent human plasma, the blood lipid levels of the recipient dogs were not far above the range of high normal for man. The fact that quantities of fatty substances localized in *injured* arterial segments in association with such blood lipid levels is in accord with the observation that in arteriosclerosis the development of lesions is not necessarily dependent upon elevated blood lipid values. However, it was found that the extent of lipid accumulations in the arteries and of xanthomatous transformation of the lesions paralleled the amount of human plasma or of lipid-rich plasma globulin given. The lesions were augmented as the levels of blood lipids in the recipient animals increased. These impressions recalled the well-known occurrence of accelerated vascular disease in persons with hyperlipemic states and also the known facts concerning the establishment of cholesterol atherosclerosis in rabbits.

In dogs receiving lactescent plasma, the respective roles of chylomicrons and of lipoproteins in the deposit of sudanophilic materials in injured arteries could not be determined. However, fatty droplets were also deposited after the injection of chylomicron-free plasma. This suggests the lipoproteins of the injected plasma as the source of these lipids. It is possible that the deposited substances may have originated in the animals' own blood, but this is considered unlikely since in control animals receiving allylamine alone there was no fatty deposit in injured arteries. Also in the

plasma or plasma-globulin injected dogs, lipids appeared in the arteries within 30 minutes to one hour after injection.

Injected as an integral part of their own clear plasma, human lipoproteins were tested for their effects on the basic allylamine lesions in as nearly a natural state as possible. However, it is uncertain whether they would react in dog arterial tissues in a way comparable to native human plasma lipoproteins penetrating the arterial walls of their own hosts. This uncertainty must apply even more forcibly to the plasma globulin fractions injected in concentrated form following separation by chemical means. In spite of these limitations, the appearance of sudanophilic material in the arterial lesions promptly after the intravenous injection of soluble, lipid-rich human globulins suggests that these large molecular substances do localize in foci of arterial injury as do also intravenously injected methylcellulose,[14] India ink,[8] or egg-yolk lipids.[18] Further, the progressive accumulation and phagocytosis of intensely sudanophilic droplets at the injured arterial sites suggests the possibility that water-insoluble fatty particles derived from the original lipoproteins were present in the lesions and stimulated the observed lipophage reactions. An investigation of factors responsible for possible denaturation of lipid-rich plasma proteins locally in the arterial wall is clearly indicated.

The precipitant action of polyethylene glycols on human plasma globulins proved convenient in the preparation of materials utilized in the present experiments.

SUMMARY

Acute inflammatory lesions of the coronary arteries of dogs were established by the intravenous injection of allylamine. Subsequent intravenous injections of chylomicron-free or of lactescent human plasma or of soluble lipid-rich human plasma globulins led to fatty deposits selectively within the basic inflammatory arterial lesions. These accumulations often occurred in association with blood lipid levels in the recipient animals near the range of high normal for man. With increasing elevation of blood lipid values, the lipid deposits in the experimental arterial lesions were augmented. Phagocytosis of the deposited lipids led to foam-cellular transformation that reproduced certain of the morphological sequences of arteriosclerosis in man. The evidence suggests that the injected plasma lipoproteins contributed to the lipid deposits at the sites of arterial injury.

A method for the separation of a lipid-rich globulin fraction of human plasma protein is described. It is based on the precipitation of the globulins by a mixture of polyethylene glycols.

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