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STUDIES ON THE PATHOGENESIS OF VASCULAR DISEASE

The Effect of a Fatty Meal on the Course of Acute Inflammatory Lesions of the Coronary Arteries and Aortas of Dogs‡

The present investigation was designed to test the effect of a single high-fat meal on the development and course of the inflammatory arterial lesions in dogs that follow a short, severe episode of hypertension. It was prompted by previous observations that intravenously injected egg-yolk lipids or lipo-proteins from human plasma modify the morphological sequences of certain experimental arterial lesions.[2, 3, 4, 5]

PROCEDURE

Healthy, mongrel dogs of varying ages and weights, previously maintained on low-fat diets, were fed a large, fat-rich meal. The meal consisted of 160 gms. of fresh egg yolk and 230 gms. of canned horse meat. The lipid content of this mixture follows (average of three samples) : total cholesterol, .65 per cent, free cholesterol, .38 per cent, fatty acids, 20.3 per cent, lipid phosphorus, 1.6 per cent. Three to five hours after feeding, during alimenary hyperlipemia, the animals' coronary arteries and aortas were injured by a short, intense episode of hypertension induced through repeated intravenous injections of epinephrine. Details of this procedure and a short description of the vascular lesions that follow it have been published.[6, 7, 8] Briefly, the mean femoral arterial pressures of dogs on experiment were maintained over a 30-minute period at near maximal levels (above 200 mm. Hg) by repeated 1 mg. intravenous injections of epinephrine (adrenalin chloride solution 1 : 1000, Parke-Davis & Co.). Usually a total of from 4 to 8 mgs. of epinephrine was administered. The animals were sacrificed at intervals after the hypertensive episode ranging from a few minutes to three weeks, and appropriate histological examination of their cardiovascular systems and viscera was carried out. In other animals, the effect of the intravenous injection of heparin upon the deposition of lipids in the arterial lesions was investigated. In all, tissues from 39 dogs were examined. Sudan IV stains of frozen sections were utilized for the demonstration of lipids, and a modified Schultz reaction for steroids. Paraffin sections were stained with hematoxylin and eosin by Masson's trichrome method or by Mallory's phosphotungstic acid-hematoxylin technique.

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In addition to the morphological investigations, plasma lipids were estimated by standard techniques in several animals before the high-fat meal and thereafter immediately preceding the epinephrine injections. Chylomicrons were removed from plasma samples by high-speed centrifugation.

TABLE 1. THE EFFECT OF A HIGH-FAT MEAL ON THE PLASMA LIPIDS OF DOGS

Plasma sample	*Total cholesterol mg.%*	*Free cholesterol mg.%*	*Lipid phosphorus mg.%*	*Fatty acids mEq./l.*
Dog No. 2039 wt. 12.0 kg.				
1. Control, fasting	131.1	32.8	11.1	9.6
2. After fat meal	161.9	55.7	14.4	21.0
3. Sample #2, chylomicrons removed	148.7	39.3	16.0	15.0
Dog. No. 2040 wt. 7.5 kg.				
1. Control, fasting	151.1	36.9	11.2	10.0
2. After fat meal	181.5	39.9	13.8	19.0
3. Sample #2, chylomicrons removed	149.4	37.8	14.0	12.0
Dog No. 2086 wt. 10.0 kg.				
1. Control, fasting	94.6	23.1	11.2	13.3
2. After fat meal	124.5	31.5	13.0	24.3
3. Sample #2, chylomicrons removed	117.8	27.7	13.5	11.6
Dog. No. 2087 wt. 11.0 kg.				
1. Control, fasting	153.6	40.3	14.4	13.3
2. After fat meal	178.5	48.7	18.0	37.0
3. Sample #2, chylomicrons removed	157.7	39.9	16.4	15.0

RESULTS

The effect of a single high-fat meal on the plasma lipids of dogs.

Table 1 gives values for the plasma lipids of four dogs before and 3-5 hours after the feeding of the standard fat meal. The large increase in fatty acids is accompanied by lesser increases in values for cholesterol and phospholipids. Changes of this nature have been observed by others. (See Brun's review.[1]) Much of the cholesterol and fatty acid excess in the blood was associated with chylomicrons and could be removed with this fraction.

Changes in the coronary arteries and aortas of control dogs given high-fat meals alone or short hypertensive episodes alone.

The coronary arteries and aortas of five dogs fed single high-fat meals failed to reveal any lesions or lipid deposits.

As previously described, lesions of the coronary arteries and aortas occurred in animals subjected to a single, epinephrine-induced hypertensive episode. As early as 10-15 minutes after the epinephrine injections, hemorrhage into the medias of scattered small muscular coronary arteries was observed. After 1-3 days, edema and necrosis of medial muscle cells was present with or without accompanying medial hemorrhage (Figs. 1 and 2). The latter was sometimes massive. Of interest was the observation that the medial damage often began in or involved only the outer smooth muscle cells lying beneath the adventitia (Fig. 3). As the lesions progressed, this distribution in many led to perivascular inflammatory reactions rather than to subendothelial proliferation (Fig. 4). Associated thrombi were present occasionally.

Also of interest were the inflammatory exudates and the phenomena of repair associated with the lesions. In many injured segments the media was packed with red blood cells with little deposit of fibrin. In these lesions there was remarkably little cellular exudate. Indeed the paucity of inflammatory cells was a notable feature in many of the injured arteries. In other vessels smooth muscle necrosis was prominent rather than hemorrhage. Here the cellular exudate was also sparse and consisted of a few polymorphonuclear leucocytes and mononuclear cells (Fig. 7). Often the exudate was confined strictly within the boundaries of the media itself, but if the damage involved the outermost medial fibers, then a mild, radially arranged adventitial and periarteritic reaction developed. Occasionally the lesions exhibited during their acute stage in the media or adventitia accumulations of a "fibrinoid" substance. There was no suggestion that this material derived from primary alteration of the collagen of these regions. The impression was definitely gained that damage to smooth muscle was the primary event. Although the medial damage in these small arteries was great, no dilatation or aneuryism formation was noted.

Healing in the lesions took place by fibrosis within 1-3 weeks. Depending on the distribution and extent of the damage, this process was medial or medial and adventitial. Only rarely did the process result in a proliferative endarteritis. The few polymorphonuclear leucocytes were replaced by scattered mononuclear cells and the necrotic medial muscle and intravascular hemorrhage by scar.

The lesions in the coronary arteries that followed epinephrine-induced hypertension were distributed both in the right and left heart. The auricular vessels were also involved. In any one artery the damage was segmental and occurred frequently in relation to branches. While the lesions were most frequent in the intramyocardial divisions of the coronary arteries, many smaller epicardial branches were involved as well.

Fat stains were carried out on a large number of lesions from control dogs maintained on a low-fat laboratory diet and subjected to the usual hypertensive episode. Occasionally in the early stages of medial necrosis a very faint diffuse sudanophilia was recognizable. The positivity of this reaction in the control lesions was so slight as never to be confused with the intense sudanophilia of the lesions of the experimental animals.

Medial necrosis and hemorrhage in the aortas of dogs subjected to hypertensive episodes occurred frequently. These aortic lesions did not differ materially from those known for over 50 years to occur in rabbits' aortas following injections of extracts of the adrenal medulla.

Changes in the coronary arteries and aortas of dogs given a high-fat meal followed by a short, severe hypertensive episode.

The tissues of 24 animals were studied at intervals from a few minutes to three weeks after a large fat meal and arterial injury. The prior ingestion of a fat meal did not alter the distribution or incidence of the lesions of the coronary arteries. The outstanding difference between the arterial lesions of the fat-fed animals and those of the controls was the accumulation in the former of massive amounts of fatty substances. Lipids were not deposited in uninjured segments of arteries.

The accumulation of lipids within the injured arteries began promptly. Sudan IV preparations of the hearts of animals sacrificed within 30 minutes after the hypertensive episode revealed diffuse, intense sudanophilia of the dogs' plasma wherever it appeared within the lumens of veins or arteries. Where hemorrhage had occurred into the walls of the arteries, the injured segments were stained diffusely and intensely with the sudan dye, exactly like the plasma within the lumens of the vessels. The suggestion was inescapable that lipid-rich plasma had entered the vessel wall at the point of injury and was responsible for the diffuse sudanophilia in these zones.

Almost immediately the histological appearance of the lipid present in the injured arterial foci began to change. Even at one hour after injury brightly staining lipid droplets and granules larger than chylomicrons began to appear in the diffusely sudanophilic medial areas. These sudanophilic droplets increased in size and number progressively up to 24-48 hours (Fig. 5), during which time the dogs' plasma as viewed histologically

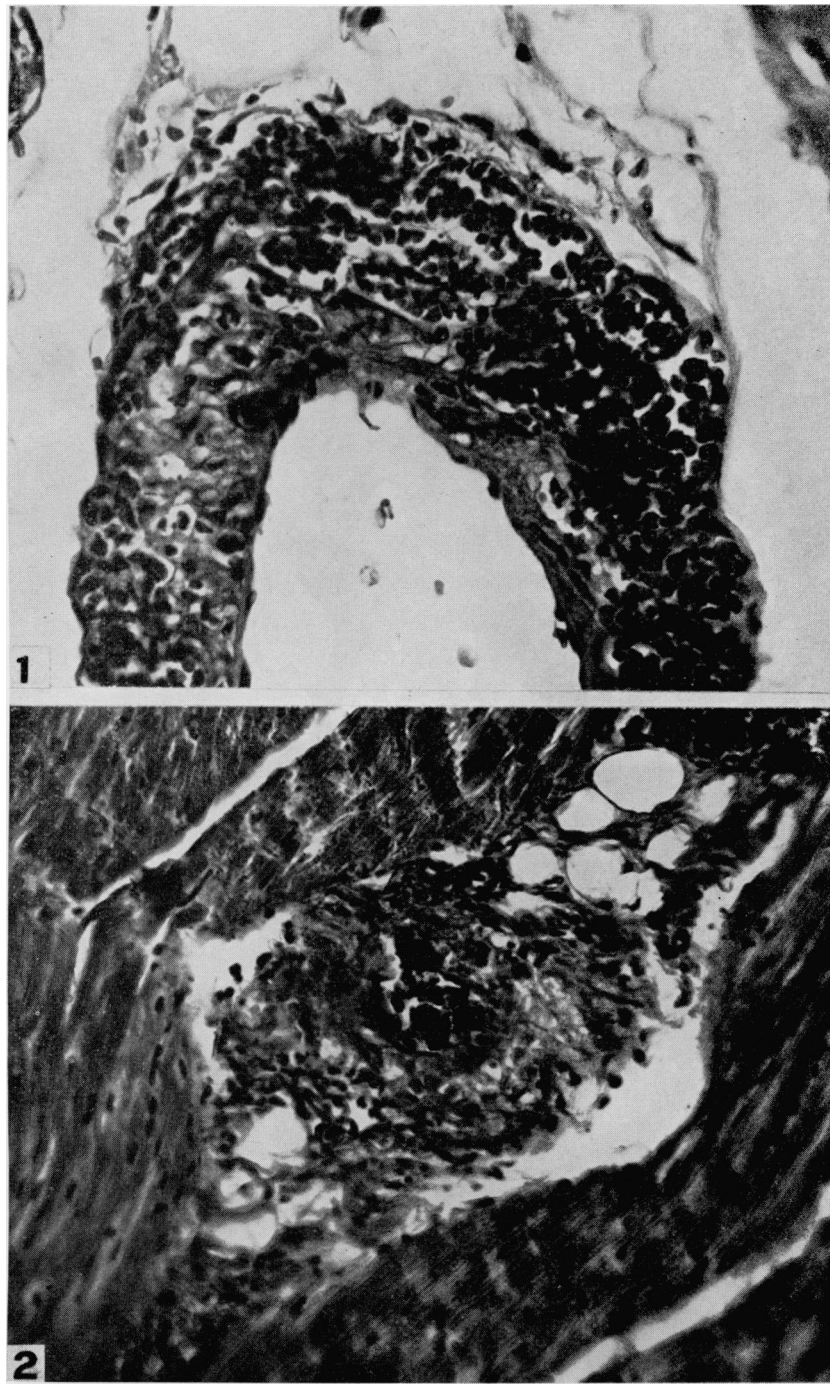


FIG. 1. Dog 1711. Cross-section of coronary artery four days after short, severe hypertensive episode. Massive hemorrhage in media. Little cellular exudate. x425.

FIG. 2. Dog 1712. Coronary arteriole 24 hours after hypertensive episode. Medial necrosis and perivascular cellular exudate. x325.

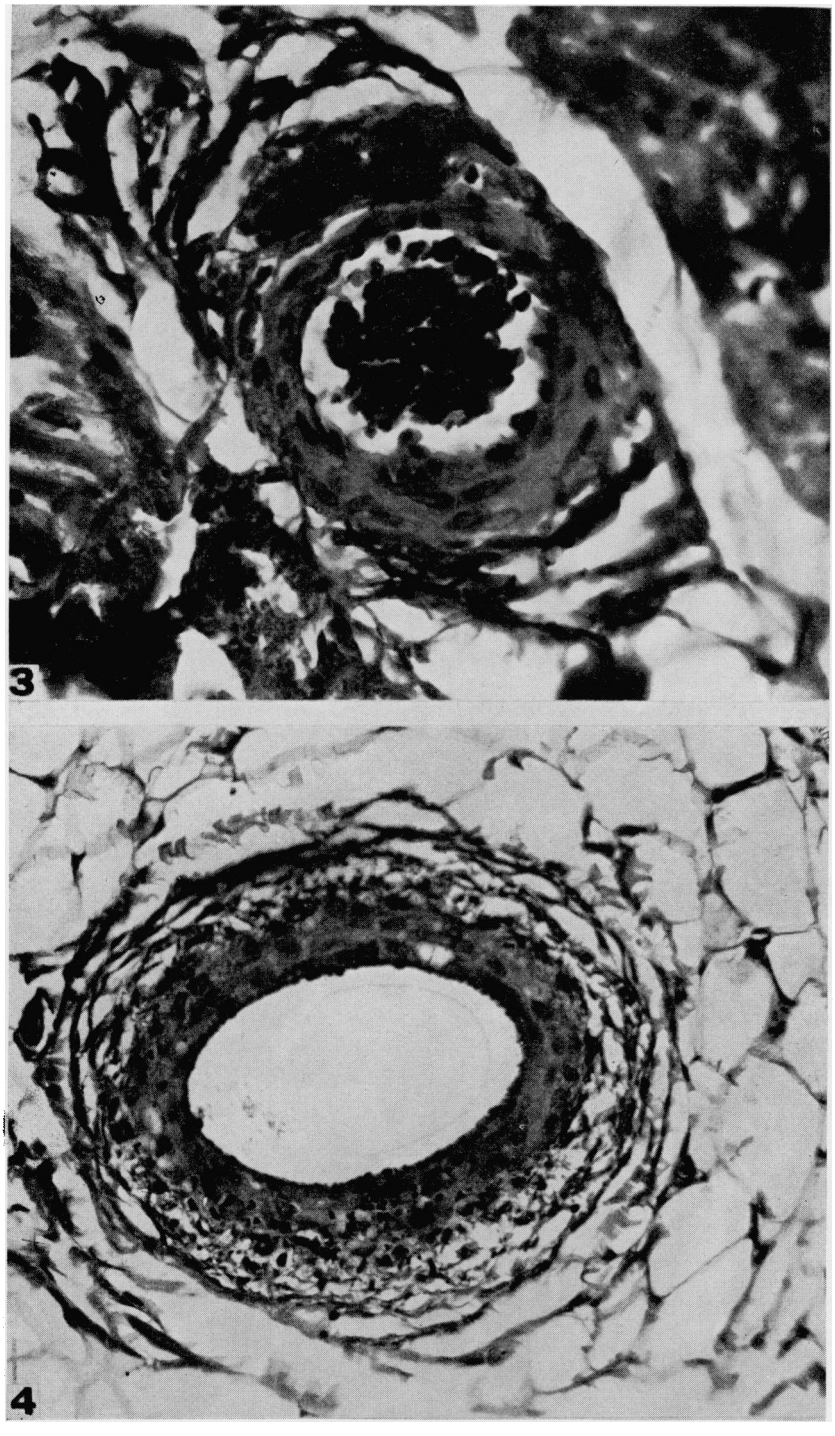
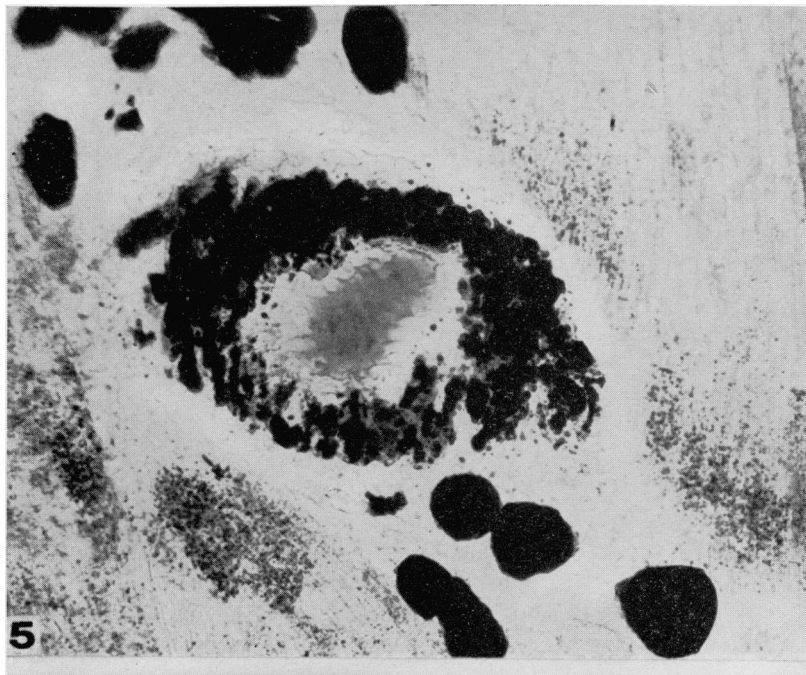


FIG. 3. Dog 1712. Coronary arteriole, 24 hours after hypertensive episode. Focal necrosis of outer media. x500.

FIG. 4. Dog 1716. Small epicardial coronary artery, six days after hypertensive episode. Necrosis of outer media with inflammatory reaction in adventitia. x325.



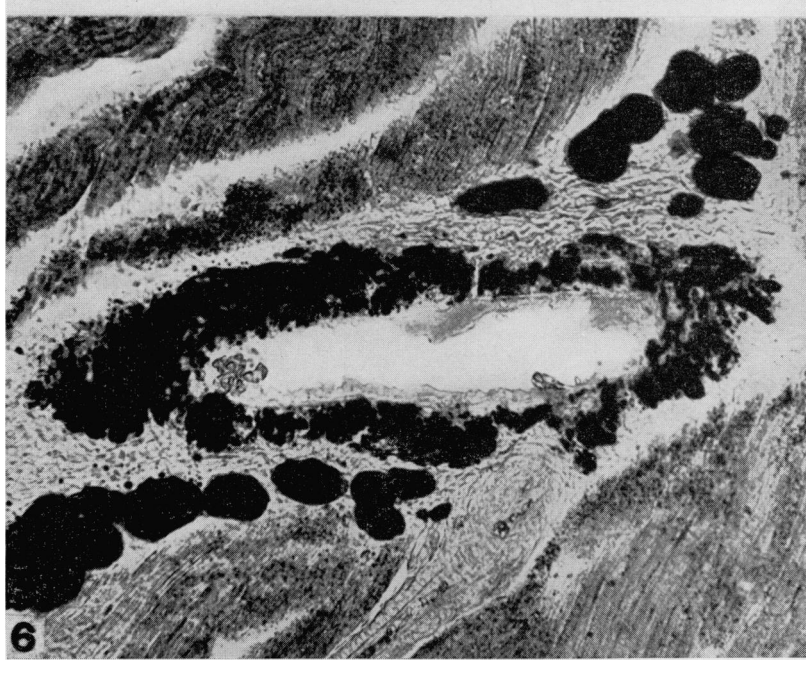


FIG. 5. Dog 2027. Coronary artery 24 hours after fat meal and hypertensive episode. Sudan IV stain, x350. Massive accumulation of lipid droplets in media. The plasma lipid stains diffusely in the lumen.

FIG. 6. Dog 1713. Coronary artery two days after fat meal and hypertensive episode. Sudan IV stain, x280. Massive deposit of lipid in media. Fat cells also are stained on either side of vessel.

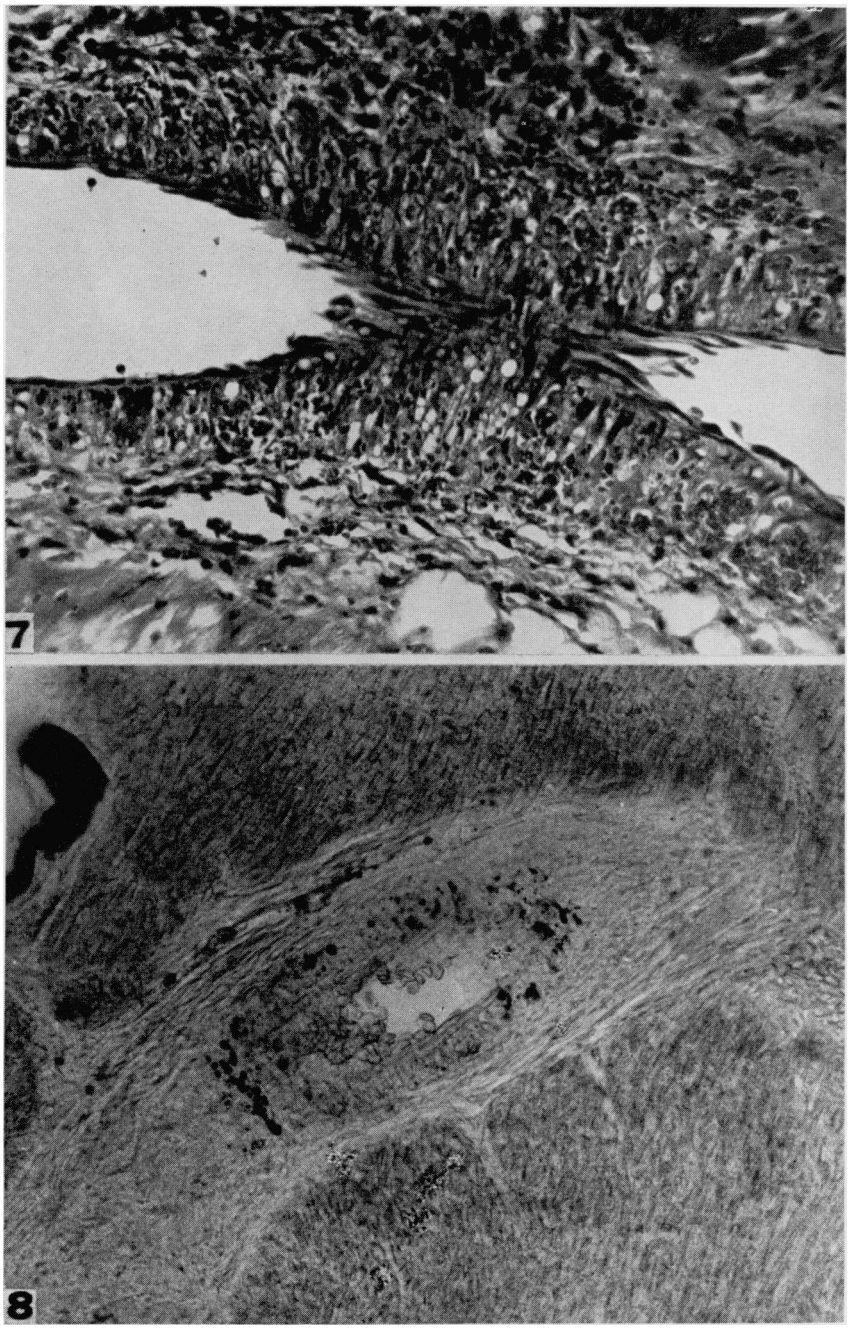


FIG. 7. Dog 1715. Tangential section of coronary artery at branch, six days after fat meal and hypertensive episode. Medial necrosis with sparse exudate. Note the numerous vacuoles representing extracellular lipid. x325.

FIG. 8. Dog 2099. Coronary artery 11 days after fat meal and hypertensive episode. Sudan IV stain, x250. Compare with Figures 5 and 6. The medial lipid has largely disappeared.

revealed a progressively decreasing diffuse sudanophilia. The lipoid droplets appeared to have accumulated and coalesced within the artery walls forming intensely staining granular masses that often obscured other medial structures (Fig. 6). The intramural lipid masses on occasion gave a faint positive Schultz reaction for steroids. In paraffin sections, routinely stained, there was little evidence of the deposited lipid in the early lesions. After several days, however, numbers of clear or partially clear medial vacuoles could be detected (Fig. 7). The presence of these relatively large amounts of lipid in the damaged media did not appreciably alter the morphological sequences already described in the lesions of control animals. After the 6th or 7th day, the lipid accumulations began to disappear (Fig. 8). The sudanophilic droplets and granular masses of lipid became progressively less and in animals sacrificed after three weeks had disappeared completely. Paraffin sections failed to reveal in the main the process by which this removal was accomplished. While an occasional monocyte with cytoplasmic vacuoles representing included lipid could be demonstrated, most of the fatty material appeared to remain extracellular in location. No foam-cellular or xanthomatous transformation of the lipid-containing medial foci occurred. The inflammatory exudates and the healing stages of the lesions were also not altered by the presence of lipids.

Lipid deposits similar to those in the coronary arteries and arterioles occurred in the injured segments of the aortas of dogs with alimentary hyperlipemia and epinephrine injections. Sequences of accumulation and disappearance of the deposited lipid followed the pattern described for the coronary arteries.

The effect of prior heparin administration on the accumulation of lipids at the sites of experimental arterial injury.

The promptness and massiveness of localization of lipids in the injured arterial walls and the absence of such deposits in lesions from control animals with nonlactescent plasmas suggested that the lipids being deposited derived from chylomicrons. It was thought of interest to determine whether or not heparin clearing of the lactescent plasma before arterial injury would prevent the accumulation of lipids in the artery wall. Consequently, six dogs were fed the standard high-fat meal. During alimentary hyperlipemia they were given 2 mgm./kg. heparin (Liquaemin sodium, Organon, Inc.) intravenously. Blood was obtained immediately after the injection and at five-minute intervals as necessary. When plasma clearing was complete, the hypertensive episode with epinephrine was instituted, followed by further blood sampling to make sure that chylomicrons did not reappear in the circulating blood. Usually they did not, and these animals

were sacrificed for histological examination from 1-72 hours after the hypertensive episode. In all of the animals hemorrhagic lesions of the small coronary arteries were present. The lipid component of the lesions was markedly altered. In no case was there accumulation of sudanophilic droplets at the sites of injury. At the earlier time intervals a diffuse sudanophilia of the lesions was present, coinciding with the increased sudanophilia of the plasma within the vessels' lumens. As the plasma staining became fainter, at longer time intervals, the sudanophilia of the lesions faded correspondingly and disappeared. No accumulations of lipid droplets or granular lipid masses were formed in the arteries.

The modification of intra-arterial lipid accumulation that followed the heparin clearing of plasma prior to injury was so striking that it prompted a test of the ability of the heparin system to clear lipid accumulations in arteries *after* injury.

The plasmas of a series of dogs were rendered lactescent by the standard high-fat meal. During the hyperlipemic state the animals' coronary arteries were injured in the usual way by injections of epinephrine. One hour following the hypertensive episode their plasmas were cleared by the intravenous injection of heparin. In some subjects of this series, complete clearing was not achieved, but in three animals the plasmas became limpid, and histological examination of the coronary arteries was carried out 1-3 hours after plasma clearing. No effect whatsoever could be detected on the lipids deposited in the injured arterial segments. In spite of the clearing of the ambient plasma, the lipid masses in the artery walls remained quite unaltered both in form and in amount.

It was of interest in contrast that clearing of lipids from the ellipsoidal arteries of the spleen occurred in the above animals. As is well known, after a high-fat meal, lipid accumulates in these fenestrated structures. Heparin clearing of the animals' lactescent plasmas either before or after epinephrine injury resulted in rapid clearing of the lipid deposits in the ellipsoids.

DISCUSSION

The foregoing experiments indicated that following a high-fat meal, massive lipid deposits occurred promptly and selectively at foci of acute injury in the coronary arteries and aortas of dogs. Intact vessels localized no lipid, emphasizing the importance of injury for this process. Evidence is presented that the accumulated lipid derived from the chylomicrons of the plasma. Thus localization of lipid did not occur in control animals with acute arterial injuries, but with nonlactescent plasmas. Further, it is known

that particulate substances, including other types of lipids,[2, 3, 4, 5, 9] accumulate, following their intravenous injection, at sites of acute arterial injury. Also, heparin clearing of the lactescent plasma before arterial injury prevented the accumulation of lipids in the vessel walls. It is unlikely that the lipid involved was plasma lipoprotein, as the content of molecularly dispersed lipid in the lactescent plasmas was essentially unchanged after the standard high-fat meal, at a time when lipid was accumulating in the arteries.

If it can be assumed that chylomicrons localized intra-arterially, it follows that these particles did not elicit phagocytic sequences in the arterial wall comparable to those occurring in arteriosclerosis in man. The lipid disappeared rapidly and apparently did not give rise to a foam-cellular xanthomatous response. This was in contrast to the fatty, foam-cellular arterial lesions that follow the combined injection of lipid-rich human plasma globulin and allylamine.[5] It must be pointed out, however, that the arterial lesions that follow allylamine injury differ from those associated with epinephrine administration in at least one important respect. The allylamine lesions usually include an extensive subendothelial proliferative component, whereas the epinephrine lesions exhibit little intimal proliferation and are mostly confined to the media. It would have been desirable to substitute allylamine injury for the epinephrine-induced hypertensive episodes in the present experiments, but this was not considered feasible because of the temporary anorexia produced in dogs by the administration of allylamine. Alimentary hyperlipemia would have been difficult to obtain during the several days needed for development of the allylamine lesions. The injured medias of dogs' small muscular coronary arteries or even the medias of dogs' aortas described in the present report cannot be considered to represent models of the intimal connective tissue of large elastic arteries in which arteriosclerosis in man develops. However, in acute arteriolar lesions in man, lipid is frequently found in the medias of the affected vessels. It is often in droplet form and is extracellular in location. Foam-cellular transformation of the media is rare. The experimental arterial lesions presented herein and the medial lipid-connective tissue reactions observed correspond more closely to certain stages of the medial lesions of human arteriolar necrosis and arteriolosclerosis than to the intimal, fatty disease of the larger vessels. Wilens has summarized well the possible relationships of these types of arterial lesions in man.[10]

Additional experiments revealed that the administration of heparin prior to the vascular-damaging hypertensive episode prevented the accumulation of lipids in the injured arterial segments. This emphasizes the importance

of the physical form of circulating lipids for their localization in the arterial wall. In the conditions of the experiment, the dispersed lipid-protein complex that is formed in heparin-cleared plasma did not localize in the arterial lesions. After the vascular injury, heparin administration, which often cleared the animals' plasma, did not effect the dispersal or removal of the aggregated lipid masses in the injured vessel wall. Apparently either the lipid particles no longer possessed the characteristics of chylomicrons, or plasma containing the clearing factor did not reach them. The ability of heparin to clear lipid accumulations from the ellipsoidal vessels of the spleen suggests that mere aggregation of chylomicrons in the vessel wall does not prevent their dispersal by the plasma-clearing system.

SUMMARY

Dogs were fed a large, high-fat meal. During the subsequent alimentary hyperlipemia, lesions of their coronary arteries and aortas were produced by a short, severe episode of hypertension induced by injections of epinephrine. Large quantities of lipids, probably derived from chylomicrons, accumulated selectively at the sites of arterial injury. This lipid did not elicit a foam-cellular phagocytic response that could be compared morphologically with arteriosclerosis in man. The lesions more closely simulated certain medial changes of arteriolonecrosis and arteriolosclerosis. The deposited lipid remained extracellular and for the most part in droplet form. It disappeared progressively from the vessel walls within two to three weeks. Clearing of the animals' lactescent plasmas with heparin before vascular injury prevented the accumulation of lipids at the subsequently injured arterial sites. Heparin administration after injury to arteries and the deposit of intramural lipids in them did not effect the removal of the fatty substances. Lipids accumulating in the ellipsoidal arteries of the spleen after a fatty meal disappeared on heparin clearing of the plasma.

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