DEPOSITION OF GLOBULAR LIPID IN ARTERIAL CELLS IN RELATION TO ANOXIA

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Intracellular deposition of visible droplets of liquid lipid is frequently initiated by anoxia. Anoxic cells lack the capacity to synthesize protein and phospholipid and therefore may not be able to emulsify and disperse the fat which they contain.[1] This paper considers anoxia as a possible determinant of fatty deposition in the intimal cells of arterial walls and records observations on the content of protein in these lipid-laden cells. A brief account is also given of some of the factors which determine fatty deposition in general, by affecting the physical state of cellular fat.

STATE OF CELLULAR FAT

In the healthy cells of many tissues, such as liver, kidney, cardiac muscle and arterial walls, fat is dispersed in an invisible state. This invisible fat may be held inside laminar micelles formed by orientated molecules of phospholipid covered by an adherent film of protein.[1] Fat in this dispersed state has neither the optical nor the solvent properties of a liquid and is masked or concealed in the cells; it may conveniently be called *micellar fat*. Cells, which in health contain no visible fat, may in illness contain droplets of liquid fat in abundance. This fat, like that of the lipocytes, dissolves the nonpolar Sudan dyes and has the physical properties of a liquid. It is not masked by dispersion, is plainly visible by microscopy as spherical droplets, and gives the tissues a creamy or fatty appearance. It is conveniently called *globular fat*.

Micellar and globular fat differ greatly in their cellular reactivity.[1] Micellar fat is the form which takes part in metabolism and transport. Globular fat is in a phase apart from these activities and must be converted into micellar fat, with which it is in equilibrium, before it can be utilized or mobilized from the cell. The dominant position of micellar fat in cellular economy is indicated in Text-figure 1.

Fatty Deposition in Disordered Cells

Deposition of visible droplets of fat inside cells consists in the transition of invisible or concealed[2] micellar fat into the visible globular form.

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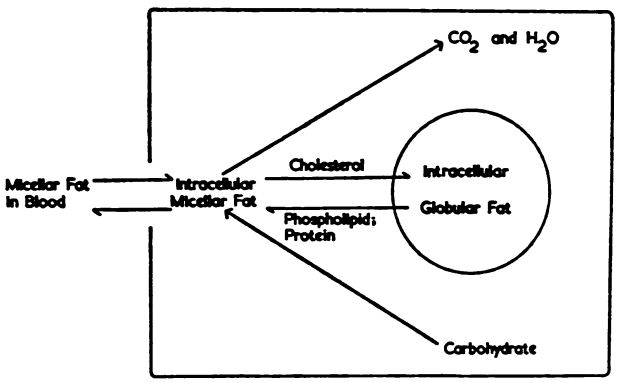
This transition occurs[1] firstly when the susceptibility to dispersion is impaired, as by deficiency in phospholipid or protein, and secondly when fat accumulates to such an extent that it cannot be held in the micellar state. Thus when, either by deficiency in emulsifying agents or by excess of fat, a certain ratio of fat to emulsifiers is surpassed, visible globular fat is aggregated or deposited inside the cell.



TEXT-FIGURE 1. Intracellular equilibrium between micellar and globular fat.

There are, thus, two basic determinants of fatty deposition in disordered cells: (a) interference with micellar dispersion of fat; and (b) increase of intracellular fat to an extent which cannot be held in the micelles.

Interference with Micellar Dispersion. Lack of phospholipid and protein,[3] which are essential constituents of the micelles, and excess of cholesterol, which antagonizes[4] the emulsifying action of phospholipid, alike cause fatty deposition by interfering with micellar dispersion.[1] Anoxia is probably an important determinant of intracellular lack of phospholipid and protein, since the synthesis of these substances is dependent on oxidative phosphorylation; anoxia is responsible for the intense fatty deposition in heart failure and anemia. Excess of cholesterol, as in diabetes, probably interferes with dispersion of fat and thus accounts for fatty change in the liver and arterial walls, so conspicuous in this disease.

Increase of Intracellular Fat. Excess of fat to an extent which cannot be held in the dispersed micellar state arises by (a) increased entry of fat into the cell, which follows excessive mobilization from the depots in starvation and wasting diseases; and (b) diminished removal of fat from the cell. This may result from defective oxidation or from defective mobilization. Actually both oxidation and mobilization may be inhibited by interference with dispersion, since micellar fat is the reactive form

which takes part in both these processes (Text-fig. 1). Thus, defective dispersion, which itself causes deposition of visible fat, also promotes increase of intracellular fat. This in turn accounts for further deposition.[1] Even when fat is not entering the cell as such, inhibition of removal causes accumulation, since fat is formed *de novo* in the cell from carbohydrate (Fig. 1).

The determinants of fatty deposition inside the cells of arterial walls are likely to be the same in principle as those outlined above for disordered cells in general. The zonal prominence of lipid-laden cells in the intima, moreover, suggests that anoxia may be an important factor in promoting deposition. Since anoxia would result in intracellular lack of protein as well as lack of phospholipid, a study was made of the content of protein in the lipid-laden cells of the intima.

Material and Methods

The location of protein and fat was studied in atheromatous plaques and lipoid spots of human arterial walls (aorta, subclavian and coronary arteries). Necropsy material from 5 patients (men aged 50 and 75, and women 54, 78 and 83 years) suffering from coronary atheroma was examined. Each piece of affected vessel wall containing lesions was divided into two parts, one of which was fixed in Carnoy's fluid and the other in formol-saline. The tissues fixed in Carnoy's fluid were then embedded in wax; sections were cut, mounted on slides without adhesive, and stained by the oxidized tannin-azo (OTA) method[5] for protein. The techniques of fixing, embedding and staining by OTA were the same as those used earlier[5] except that the time of immersion in cold buffered diazotized o-dianisidine (after tanning and oxidation) was increased from 10 to 20 minutes. Sections of the tissues fixed in Carnoy's fluid were also stained by hematoxylin and eosin. Fat was demonstrated in the tissues fixed in formol-saline: for this purpose, frozen sections were cut and stained with a mixture of Sudan III and Sudan IV (saturated solution of the two dyes in 70 per cent ethanol), Carazzi's hematoxylin being employed as a counterstain. Frozen sections of lesions in 9 different vessels and wax sections from lesions in 8 different vessels were examined after staining by the various techniques employed.

Results

Fusiform, stellate or spherical cells filled with droplets of lipid were visible in the intima in all the arterial lesions examined (Figs. 1 and 2). These large fatty cells were confined to the intima; they abutted the internal elastic lamina in some of the lesions, but subendothelial and mid-intimal aggregations of fatty cells were also encountered. Only very occasional minute fusiform fatty cells were visible in the media. Extracellular and intrafibrillar droplets of fat were also seen; this fat occurred in finer droplets and appeared to be more diffuse than the intracellular fat. Moreover, some of the extracellular and intrafibrillar droplets were present in the media as well as in the intima, although the intimal deposits were much more substantial. Intracellular fat was almost entirely restricted to the intima.

With the OTA method the intimal fatty cells appeared foamy, and their cytoplasm contained little tannophilic protein (Figs. 3 to 6). In contrast, the surrounding collagen and vascular endothelium were rich in protein stainable by OTA. The internal elastic lamina (Figs. 5 and 6) and the elastic fibers of the media were uncolored by OTA; the rest of the media was intensely stained. Extracellular droplets of lipid appeared as foamy material only faintly colored by OTA. The foam cells with scanty protein were entirely intimal in location. In one lesion, foam cells rich in protein were seen around extravasated plasma.

Discussion

The purpose of this paper is not to consider the cause of atheromatous thickening which has been discussed so fully in recent years,[6-8] but to focus attention on the process of fatty deposition within the intimal cells.

An admirable account of the cellular deposition of visible lipid in arterial walls was given by Virchow.[9] He emphasized that droplets of lipid first appeared inside the cells of intimal connective tissue: the fatty cells occurred both in superficial whitish spots (*weissliche Flecke*) and in the depths of atheromatous foci (*Heerde*). Hueper[7] suggested that there was, indeed, no fundamental distinction between the genesis of these lipid spots and the more deeply situated atheromatous plaques; at any rate connective tissue cells bloated with droplets of liquid lipid and containing scanty protein occur in both types of lesion (Figs. 3 and 6). The cells containing the fatty droplets may be elongated (Fig. 1), stellate or spherical (Fig. 2); they thus vary from the spindle-shaped and stellate elements (*spindel-oder sternförmig*) to the spherical granular cells (*rundlichen Körnchenzellen*) so graphically described by Virchow.

The determinants of fatty deposition in these intimal cells are probably very similar to those responsible for intracellular fatty change in general. Indeed, Virchow[9] regarded the initial fatty change (*Fettemetamorphose*) in intimal cells as in no wise different (*absolut nicht verschieden*) from fatty alterations which he had already described in the cells of other tissues. Recently, Geer, McGill, Strong and Holman,[10] using electron microscopy, found droplets of fat in the muscle cells of atheromatous arteries, and they suggested that fat was formed as the result of metabolic processes in the cells.

The most remarkable feature of fatty change in the cells of arterial walls is its zonal character. The usual outer limit of intense intracellular fatty deposition is the internal elastic lamina. Outside this membrane, deposition is absent (Fig. 1) or much less intense. In atheromatous plaques the cells containing the most abundant droplets of lipid are often

situated in the outer intima just internal to the elastic lamina. This zonal disposition of fatty cells, moreover, indicates that the droplets of fat have an intracellular origin and that they do not arise by ingestion of extracellular deposits which would form in a more random manner. Extracellular lipids may arise from disintegration of lipid-laden cells.

There is, indeed, a striking resemblance between the zonal distribution of lipid-laden cells in arterial walls and the zonal limits of visible lipid in the liver and heart when globular fat appears in these organs. The deposition of fat in hepatic lobules is frequently determined by relative anoxia of the centrally situated cells; in these cells fatty deposition is most prevalent, since in them the relatively low tension of oxygen probably interferes with synthesis of protein and phospholipid necessary for the dispersion of fat. Similarly, in the heart, the muscle fibers close to the cavities are those most prone to anoxia, and these fibers are also most affected by fatty deposition in disease.[11]

It seems likely that relative anoxia alone determines the zonal distribution of visible lipid in arterial disease. In normal arterial walls the vasa vasorum supply the adventitia and penetrate the media; but the intima is usually devoid of blood vessels. Hueper [6] supposed that arteriosclerosis could be caused by ischemic hypoxia, which interfered with the oxidative metabolism and nutrition of the vascular wall and was followed by increased permeability with infiltration by constituents of the plasma. Dible [12] also emphasized that the deeper layers of the intima were deficient in vascular supply and designated the internal elastic lamina as a barrier to vascularization of the intima. It is now suggested that ischemic anoxia is the principal determinant of fatty deposition within the intimal cells.

Defective oxygenation may cause fatty deposition by interference with the synthesis of protein and phospholipid. Both of these substances are necessary for the micellar dispersion of fat, and their synthesis is dependent on oxidative phosphorylation within the cell. Phospholipid appears to be absent from the hydrophobic lipids of intimal macrophages.[18] The observations recorded above show that the cytoplasm of the fatty intimal cells contains hardly any tannophilic protein (Figs. 3 to 6). This lack of protein is not a universal characteristic of all fatty cells, since abundant tannophilic protein is present in fatty hepatic cells of rats poisoned by carbon tetrachloride (unpublished observations). Loss of protein is therefore not an obligatory sequel to fatty deposition. Many of the intimal fatty cells, indeed, contain so little protein that it seems reasonable to suppose that lack of protein interferes with micellar dispersion and is thus the determinant of the deposition of globular fat. Moreover, the transition of micellar to globular fat (Text-fig. 1) would

interrupt utilization and so promote accumulation of excessive fat which may in turn lead to further deposition.

Interference with dispersion may even promote accumulation when no fat is entering the cell as such, since fat may be formed *de novo* in the cell from carbohydrate (Text-fig. 1). This cycle of events initiated by anoxia would thus result in the formation of lipid-laden cells containing scanty protein which are confined to the intima.

This view of the deposition of globular lipid in the intimal cells accords well with the conditions which are known to predispose to atheroma. Firstly, in the aging or hypertensive patient, narrowing of the vasa vasorum and reduplication of the internal elastic lamina would tend to increase intimal anoxia and so cause intracellular deficiency in protein and phospholipid. Secondly, the attachment of mural thrombi [14,15] and also subsequent organization [15] would impede oxygenation of the intimal cells and so promote deposition. Finally, in diabetes, excessive amounts of cholesterol may antagonize the emulsifying action of phospholipid,[4] and this would be likely to affect most severely the intimal cells, where lack of oxygen diminishes the available phospholipid and protein. In all these instances interference with intracellular dispersion of fat may thus herald fatty deposition in the intimal cells.

Summary

Fatty deposition or aggregation consists in the appearance of visible droplets of liquid lipid in disordered cells which in health contain no visible fat. This change involves the transition from micellar fat, which is dispersed and invisible in the cell, into globular fat, which has all the physical properties of a liquid lipid. Fat appears as visible droplets firstly, when its susceptibility to dispersion is impaired, and secondly, when it accumulates to such excess that it can no longer be dispersed completely by phospholipid and protein in the micellar form. Moreover, since undispersed globular fat cannot be oxidized or mobilized from the cell, interference with dispersion may itself promote accumulation and thus herald further deposition of visible droplets of fat. Thus, deposition may occur even when no fat is entering the cell, since fat can be formed within the cell from carbohydrate.

Protein and phospholipid are essential intracellular emulsifiers for the dispersion of fat in the micellar state. Lack of these substances within the cells thus promotes deposition of globular fat. Since intracellular oxidative phosphorylation is necessary for the synthesis of both protein and phospholipid, anoxia is likely to be a basic determinant of fatty deposition.

In arterial walls the cytoplasm of lipid-laden cells (in atheromatous

plaques and lipoid spots) contains but little tannophilic protein. It is suggested that this intracellular lack of protein coupled with lack of phospholipid is responsible for the appearance of fat.

Lipid-laden cells with scanty cytoplasmic protein are confined to the intima of arteries. This zonal distribution of fatty cells in the most anoxic region of the arterial tissue suggests that anoxia may be the underlying cause of lack of protein and lack of phospholipid, which in turn lead to fatty deposition in atheroma.

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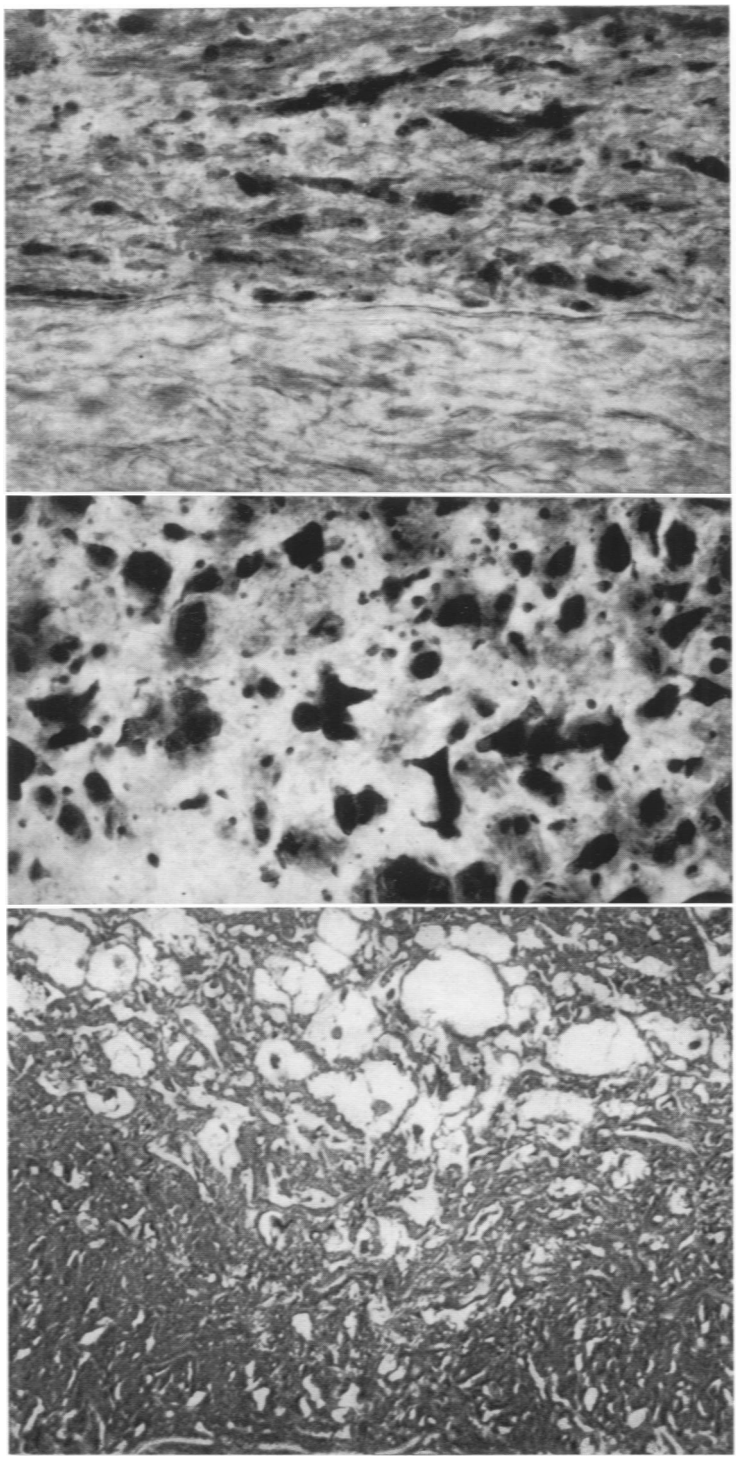
[*Illustrations follow*]

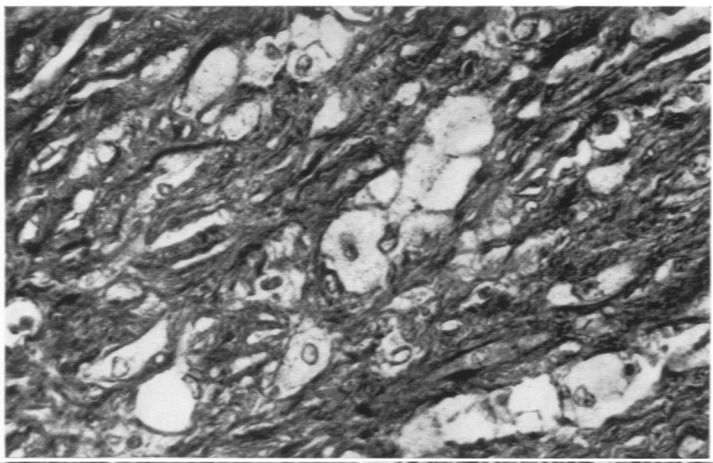
Legends for Figures

Fig. 1. Elongated intimal cells situated inside the internal elastic lamina (which crosses the field) are filled with droplets of globular lipid stained by Sudan III and IV. The media outside this lamina contains no fatty cells. Subclavian artery. ×400.

Fig. 2. Stellate and spherical intimal cells are filled with globular lipid stained by Sudan III and IV. Coronary artery. × 400.

Fig. 3. Empty-looking intimal cells are stained for protein by the oxidized tannin-azo (OTA) method. These cells are deeply situated in thickened intimal connective tissue. The internal elastic lamina is visible at the lower edge of the field. This is the same vessel shown in Figure 2. × 400.





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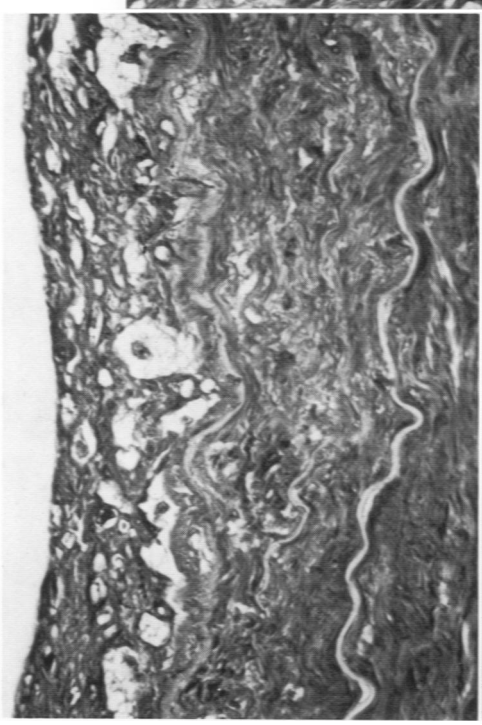

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FIG. 4. Intimal fatty cells are stained for protein by OTA. These cells are embedded in a thickened intimal connective tissue. Their cytoplasm contains only traces of tannophilic protein. This is the same vessel shown in Figure 1. × 400.

FIG. 5. Scattered subendothelial fatty cells are stained for protein by OTA. There is little tannophilic protein in the cytoplasm of these intimal cells; but the connective tissue, except for the internal elastic lamina, contains abundant tannophilic protein. Same vessel as shown in Figure 1. ×400.

FIG. 6. A zone of more numerous subendothelial fatty cells stained for protein by OTA. Their cytoplasm is conspicuously lacking in tannophilic protein. Same vessel as shown in Figure 1. × 400.