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THE ORIGIN OF FOAM CELLS IN ATHEROSCLEROSIS

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SUMMARY.—The origin of the foam cells of experimental atherosclerotic lesions in rabbits has been investigated. Light and electron microscopic techniques were used.

Lesions were induced by comparatively low elevations of serum cholesterol (up to 550 mg./100 ml.) and maintained in a narrow range (450–550 mg./100 ml.) by dietary manipulation.

Two types of foam cell are described. One of these is considered to be a modified smooth muscle cell derived from the cells of the arterial media. The second is a macrophage of reticuloendothelial origin probably a blood monocyte.

The smooth muscle foam cell is released into the developing plaque by destruction of the elastic laminae to which these cells are normally attached. Active migration of cells through intact or split laminae was not seen.

Following assimilation of these cells into the plaque, modifications occur involving predominantly the cell ergastoplasm.

Such modifications are considered to reflect activation of these cells to form collagen and elastic fibres in an attempt to regain their resting cell-fibre relationships and repair the damage to the arterial wall.

The macrophage foam cell is an extremely active phagocyte which rapidly achieves large size and then degenerates. The cellular debris from this degeneration forms a considerable portion of the gruel core of older plaques.

It is suggested that measures to promote the activities of the smooth muscle cell or to inhibit those of the macrophage could be of considerable importance in encouraging healing of atherosclerotic lesions and restoration of normal function to the damaged arterial wall.

AT some stage in their development, all atherosclerotic lesions contain foam cells. The characteristic feature of such cells is the large number of lipid filled vesicles in their cytoplasm. In paraffin sections the lipid is removed and under the light microscope the cytoplasm now has a foamy appearance from which these cells are named. In experimentally induced lesions foam cells form the bulk of the lesion particularly during the early stages of its development. In spontaneously occurring lesions their presence in the plaque may be preceded by a prelipid stage of intimal injury but whether this is so or not and there is some dispute on the point, there is no doubt that foam cells eventually make their appearance in the lesions.

However, despite their prominance there is still considerable controversy as to the exact origin of these cells. In the literature two contrasting points of view are found almost equally represented. The first of these holds that foam cells develop from the smooth muscle cells of the arterial media. Such cells are thought to migrate into the intima through breaks in the internal elastic lamina. They

may proliferate by division and accumulate lipid either by phagocytosis, synthesis, or diminished utilization in the face of increased uptake. Furthermore these cells are said to form the collagen and elastic fibrils found in advanced plaques. This concept of the smooth muscle origin of foam cells was first developed by Altschul (1950*a*). Recently, amongst others, it has been supported by Parker (1960); Haust, More and Movat (1960); Buck (1962); Luginbühl and Jones (1965); Parker and Odland (1966); Wissler (1968) and vigorously by Constantinides (1965).

The second viewpoint maintains that most foam cells are derived from macrophages of which the majority are blood borne cells probably monocytes or conceivably lymphocytes. Fundamental to this idea is the concept that the initial lesion in atherosclerosis involves an accumulation of lipid in the arterial intima. Such an accumulation acts as an irritative focus attracting the wandering histiocytes from the blood. In recent literature this view has been supported by Gonzales (1963); Still and Marriott (1964); Geer and Guidry (1964); Geer (1965*a*, *b*); Gonzalez and Furman (1965); Marshall, Adams, O'Neal and Debakey (1966); Lindsay and Chaikoff (1966) and significantly by Anitschkow (1967).

It should be noted that a few workers present concepts which are to some extent a compromise between the two contrasting views discussed above. Baylis, Hurst and More (1964), French and Jennings (1965), Imai, Lee, Pastori, Panlilio, Florentin and Thomas (1966) and Boelsma-Van Houte and Bottcher (1967) either state or indicate in their illustrations that foam cells may have a dual origin either from smooth muscle cells or from wandering macrophages. Also in a recent study Constantinides (1968) appears to have modified his rather strong position on this subject and to admit the possibility of such a dual origin.

The studies quoted are a pertinent selection from what is now a vast literature and cover not only experimental lesions but also naturally occurring human and animal lesions.

Hence a striking difference of opinion exists as to the nature of the cell which is of the most fundamental significance in the atherosclerotic process.

This paper presents my own findings on this subject in both light and electron microscopic studies of experimentally induced lesions in rabbits.

In the experiments described lesions induced by low, up to 550 mg./100 ml., but constant levels of hypercholesterolaemia were studied. That such levels of cholesterol elevation will cause suitable lesions if maintained for sufficient periods of time has been demonstrated consistently in my previous studies of the effects of alfalfa feeding on experimental hypercholesterolaemia (Cookson, Altschul and Federoff, 1967; Cookson and Federoff, 1968). Thus an often quoted objection that the degree of hypercholesterolaemia required in experimental cholesterol atherosclerosis is excessive was nullified. Furthermore, in my experience, lesions induced in this manner appear to resemble naturally occurring human lesions more closely in their pathogenesis.

MATERIALS AND METHODS

Mature rabbits were used as experimental animals. Both sexes were used without concern, for our previous experience and that of other workers (Altschul, 1950*b*) has demonstrated that individual variations in response to cholesterol administration are of greater significance than variations which can be attributed to sex.

All the animals were given 0·4 g. of cholesterol by mouth daily in gelatin capsules.

On 3 consecutive occasions before the start of cholesterol administration and throughout the experimental period, the serum cholesterol level of each animal was estimated at weekly intervals using the method described by Bowman and Wolf (1962).

The resulting elevation of serum cholesterol level was then controlled in a range of 450–550 mg./100 ml. throughout the experimental period by varying the alfalfa content of each animals diet in the light of the individual response. The mechanism of achieving this control has been developed from previous experiments on the effects of alfalfa feeding on cholesterol induced hypercholesterolaemia (Cookson *et al.*, 1967; Cookson and Federoff, 1968). For such control in the present experiments 2 diets were used. One containing 25 per cent alfalfa, the other 50 per cent alfalfa. The detailed composition of these diets is given in Table I.

TABLE I.—*Composition of Diets*

	Weight (lb.)
(1) Standard calf meal diet*	
Oats No. 1	350·0
Wheat	460·0
Soya bean meal	100·0
Calcium phosphate	9·0
Vitamin A premixed	1·0
Vitamin D premixed	0·1
Vitamin E premixed	2·5
Iodized salt	5·0
Beet molasses	50·0
Brewers yeast	20·0
Total	997·6
(2) 25 per cent experimental alfalfa diet*	
Alfalfa	25
Standard calf meal diet	75
Total	100
(3) 50 per cent experimental alfalfa diet*	
Alfalfa	50
Standard calf meal diet	50
Total	100

* Mixtures were made up into pellet form.

The animals were divided into groups with respect to the duration of the experimental period, that is the length of time to which they were subjected to hypercholesterolaemia.

The first group had an experimental period of 6 months, the 2nd 9 months and 3rd 12 months. Animals dying, refusing to feed or suffering from intercurrent disease were replaced in the experimental groups.

The experiments were continued until tissues from 10 animals in each group had been obtained.

At the end of their allotted time, the animals were killed. From each the entire aorta was removed and cut into pieces of about $\frac{1}{4}$ in. in length. From these suitable samples were taken and processed for microscopical examination by one or more of the following methods:

1. Standard histological techniques for light microscopy including haematoxylin and eosin, periodic acid Schiff and fat stains such as Sudan Black B, Sudan IV and Oil Red O.
2. Histochemical techniques on frozen sections in particular Burnstone's technique for acid phosphatase using Naphthol AS–BI phosphate as substrate with the tissue fixed overnight in cold 15 per cent neutral buffered formalin.
3. A silver impregnation technique devised by Marshall (1956) and using frozen sections of tissue fixed in cold 15 per cent neutral buffered formalin. Many workers consider this technique specific for macrophages of reticulo-endothelial origin. Positively reacting cells have a strongly metalophilic cytoplasm.
4. Standard electron microscopic techniques with the tissue fixed in 5 per cent glutaraldehyde, post fixed in 1 per cent osmium tetroxide, embedded in epon, sectioned and stained by the combined lead citrate, uranyl acetate method.

RESULTS

Atherosclerotic lesions of differing age, size and degree of development were found in the aortas of all the animals involved. Their distribution follows the usual pattern described by most workers in this field.

In essence, these lesions may be said to have a cellular and an extra-cellular phase. The latter consists of collagen and elastic fibres and fibrils, ground substance, lipids, crystals, cell debris and so on, and this component varies widely in composition and extent of organization in different plaques. However, it is with the cellular elements of the lesions that I am concerned in this paper and the qualitative observations on these were extremely consistent independent of the size, age or degree of development of the plaque.

Apart from the covering endothelium, 2 types of cells both of which are foam cells were found. Significant results as to the nature and origin of these cells were obtained from the electron microscopy studies, the silver impregnation method of Marshall and histochemical studies for acid phosphatase.

The first type of foam cell may be best described as a modified smooth muscle cell. Found throughout the lesions these cells vary considerably in size and shape but show all the common features of arterial smooth muscle cells. In addition they have several other features, mainly cytoplasmic and developed in varying degree but which justify calling them modified.

Typical examples of illustrative portions of these cells are shown in Fig. 1–6 and 12, all of which are electron micrographs.

The morphological characteristics which identify this type of foam cell as of smooth muscle origin are: 1. a continuous surrounding basement membrane; 2. typical surface pinocytotic vesicles; 3. cytoplasmic fibrils, usually best seen in the peripheral cytoplasm; 4. dense bodies within this fibrillar cytoplasm; 5. a morphologically typical nucleus; 6. in the early stages of their development as foam cells, a grouping of their other cytoplasmic constituents, such as mitochondria and endoplasmic reticulum, in a perinuclear situation.

With the electron microscope I have never seen evidence of mitotic division in these cells and whilst degeneration and breakup is occasionally observed it is relatively rare in comparison to the frequency of such changes seen in the second type of foam cell.

The cytoplasmic modifications of these smooth muscle foam cells involve both organelles and inclusions. As already indicated droplets of lipid appear in the cytoplasm with the number, size and distribution of these varying from cell to cell. These droplets are best described as occupying cytoplasmic vacuoles rather than vesicles for they seldom appear to be membrane bound (Fig. 1, 4, 5 and 6). In addition to these lipid inclusions marked changes in the ergastoplasm are evident. As shown by Fig. 1–5 and in comparison to normal arterial smooth muscle cells, specifically those of the tunica media, there is a very considerable increase in the content of rough surfaced endoplasmic reticulum and free ribosomes. There is an increase in the size and number of mitochondria and an increase in the extent and degree of development of the golgi complex (Fig. 3). The rough surfaced endoplasmic reticulum appears to have a functional relationship to the lipid vacuoles for one often has the impression that the somewhat dilated cisternae of the reticulum may open into them (Fig. 5).

Finally these cells only rarely show evidence of phagocytic activity. In

addition residual bodies, myelin figures or other evidence of prominent lysosomal action are not common.

The second type of foam cell is very different from that described above. They are found in all but the very smallest of lesions and they form the bulk of the florid, almost pure foam cell, plaques which were occasionally encountered in these animals but which are more typical of this disease when induced by very high levels of serum cholesterol.

EXPLANATION OF PLATES

FIG. 1.—Portion of cytoplasm of a smooth muscle foam cell. The basement membrane (B), fat vacuoles (V), increased rough surfaced endoplasmic reticulum, and mitochondria (M) are easily identified. × 8100.

FIG. 2.—A higher power micrograph of the central portion of the main cell in Fig. 1. In addition to the basement membrane (B), rough surfaced endoplasmic reticulum (E) and mitochondria (M), other features can be seen. These include free ribosomes (R), surface pinocytotic vesicles (P) and myofibrils with associated dense bodies (D) in the peripheral cytoplasm. × 35,000.

FIG. 3.—Cytoplasmic process of a typical smooth muscle foam cell. Characteristic features well demonstrated in this figure include the basement membrane (B), rough surfaced endoplasmic reticulum, agranular golgi profiles (G), the fibrillar peripheral cytoplasm with dense bodies and surface pinocytotic vesicles (P). × 35,000.

FIG. 4.—Portions of the cytoplasm of three smooth muscle foam cells. Many of the features noted in Fig. 1–3 can be seen and the centre cell shows the typical extensive development of the rough surfaced endoplasmic reticulum and free ribosomes particlarly well. × 27,000.

FIG. 5.—Another ρortion of the cytoplasm of the cell shown in Fig. 4. The fat vacuoles (V) are usually found within the increased ergastoplasm and often the distended cisternae of the reticulum appear to be opening into them (arrow). × 24,000.

FIG. 6.—A transverse section of a smooth muscle foam cell through the nucleus (N). The fat vacuoles (V) are prominent and other distinguishing characteristics noted in previous figures can be made out. × 8400.

FIG. 7.—Portion of a macrophage foam cell including both cytoplasm and nucleus (N). Within the cytoplasm numerous fat vacuoles and primary and secondary lysosomes are evident. Some of the primary lysosomes are identified (arrows). The margin of the cytoplasm shows phagocytic tentacles (t) and forming phagocytic vesicles (pv). There is no surrounding basement membrane to the cell. × 4100.

FIG. 8.—Higher power micrograph of the cell margin in Fig. 7 at which active phagocytosis and the formation of phagocytic vesicles (pv) is evident. × 27,000.

FIG. 9.—Nucleus (N) and surrounding cytoplasm of a macrophage foam cell. The nucleus is pyknotic and the cell is breaking up. × 6000.

FIG. 10.—Another example of degeneration and breakup in a macrophage foam cell. Although the nucleus (N) appears to be reasonably healthy the cytoplasm can be seen to be disintegrating. × 7000.

FIG. 11.—Macrophage foam cell lying immediately beneath the endothelium (En) of the plaque. The cell nucleus (N) is in mitosis. × 6800.

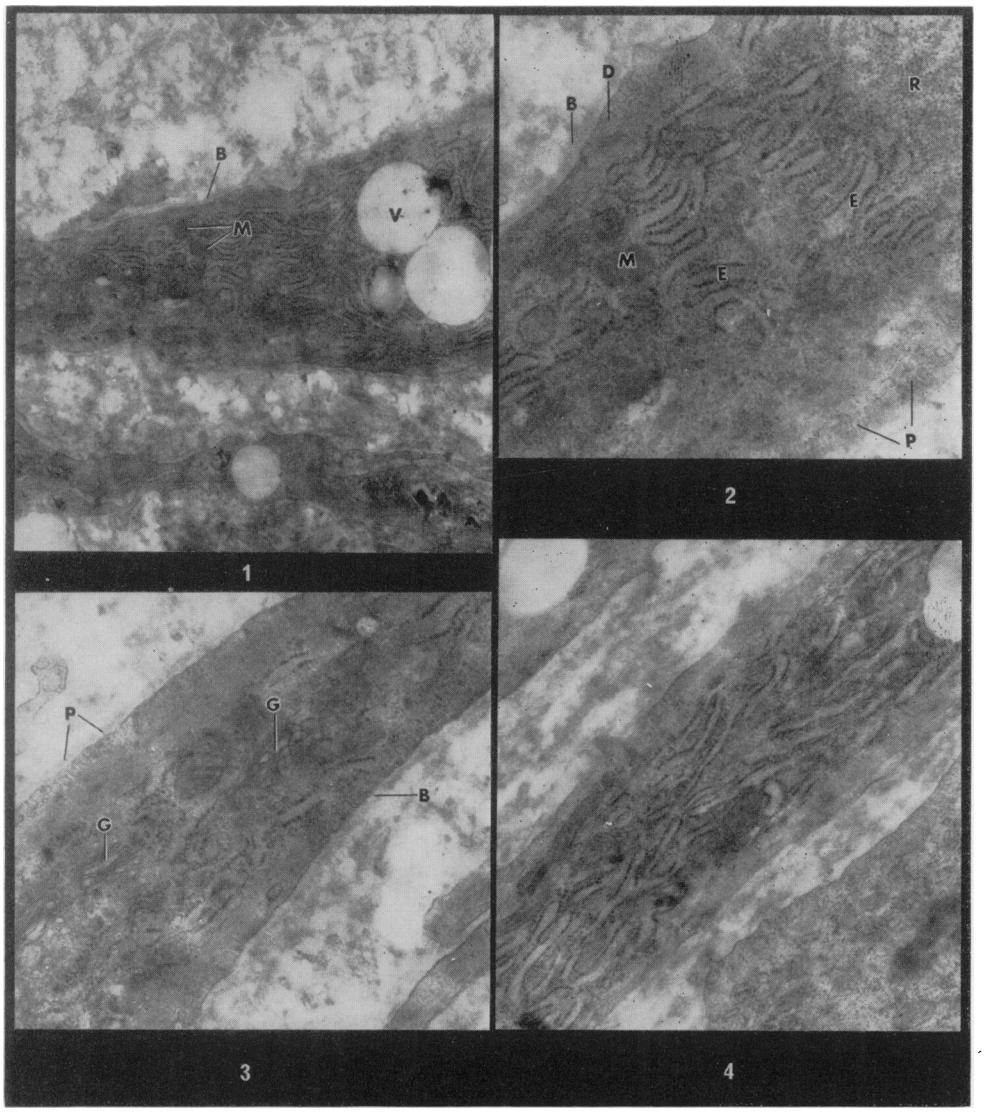
FIG. 12.—Micrograph showing portions of both macrophage and smooth muscle foam cells. Cell 1 is a macrophage foam cell. Cells 2 and 3 are smooth muscle foam cells. The characteristic features of both types of cell are evident as is the contrast between them. × 15,700.

FIG. 13.—Metalophil macrophages. Early uniformly impregnated cells are confined to the superficial layers of the plaque. Even these show evidence of cytoplasmic vacuolation. Four cells are indicated (arrows). Marshall's stain. × 330.

FIG. 14.—Metalophil macrophages. Two early forms are indicated (arrows). A third cell (f) is a more advanced foam cell with the metalophil cytoplasm being reduced to a peripheral rim. Marshall's stain. × 330.

FIG. 15.—Metalophil macrophages. The cell indicated (arrow) is almost a fully developed foam cell. Metalophil cytoplasm is seen as a peripheral rim and around the nuclear region. Marshall's stain. × 830.

FIG. 16.—Small plaque showing the typical reaction to the stain for acid phosphatase. The most intense reaction is mainly confined to the more superficial layers of the plaque. In the deeper part isolated reacting cells which are foam cells can be seen. An example is indicated by the arrow. Burnstone's method. × 210.



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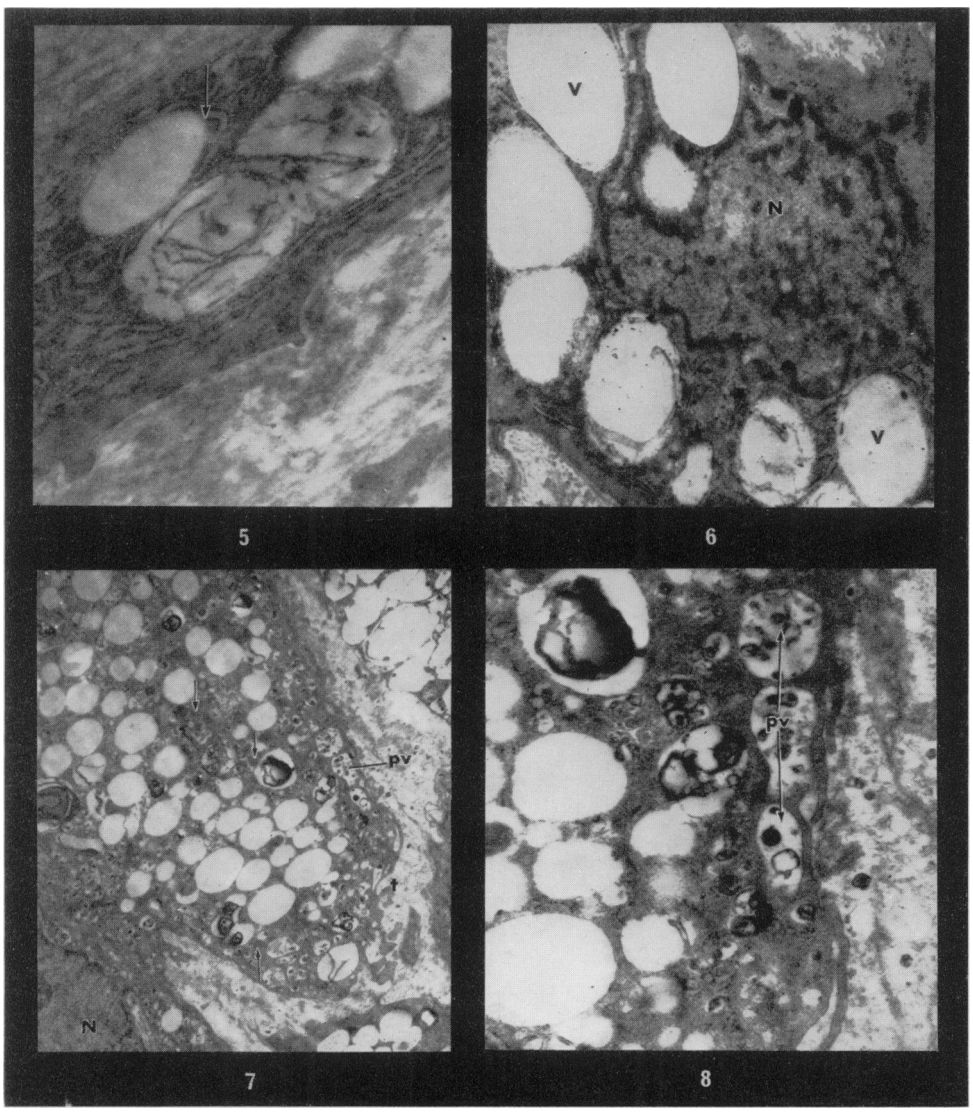
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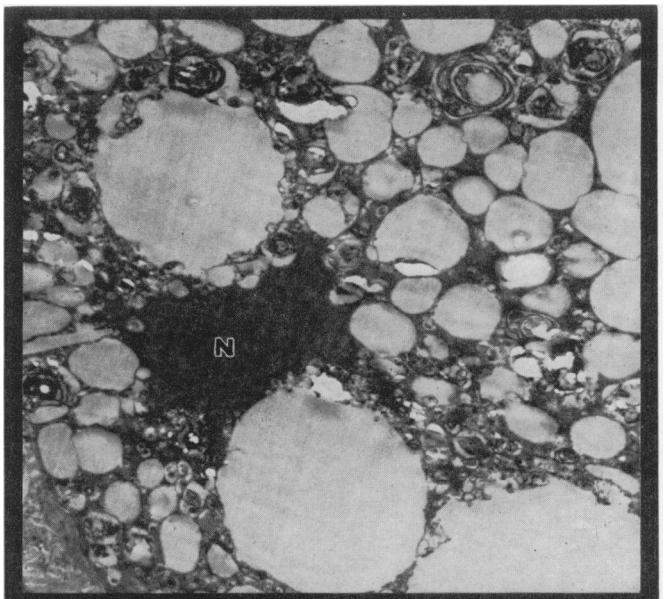

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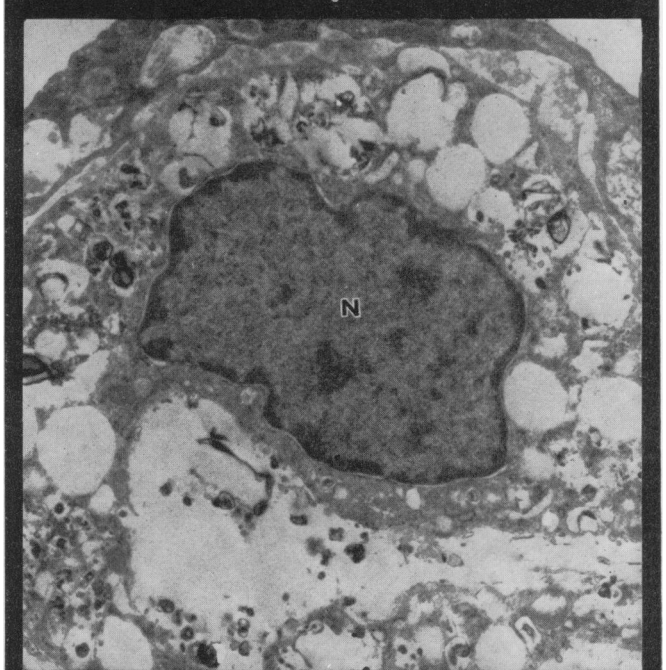

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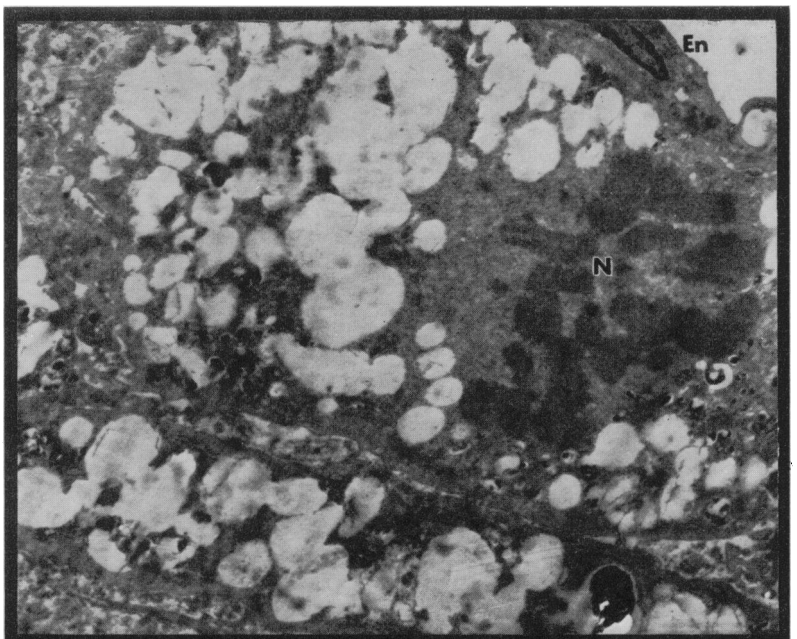

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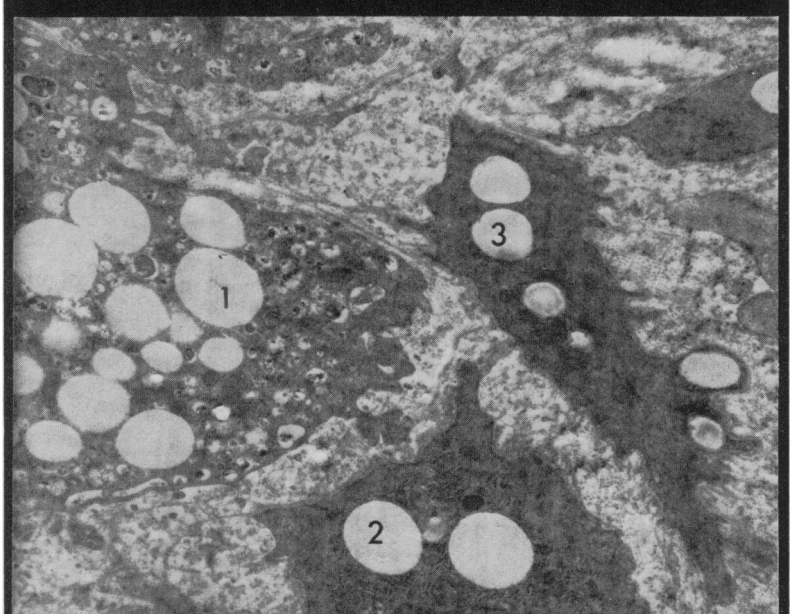

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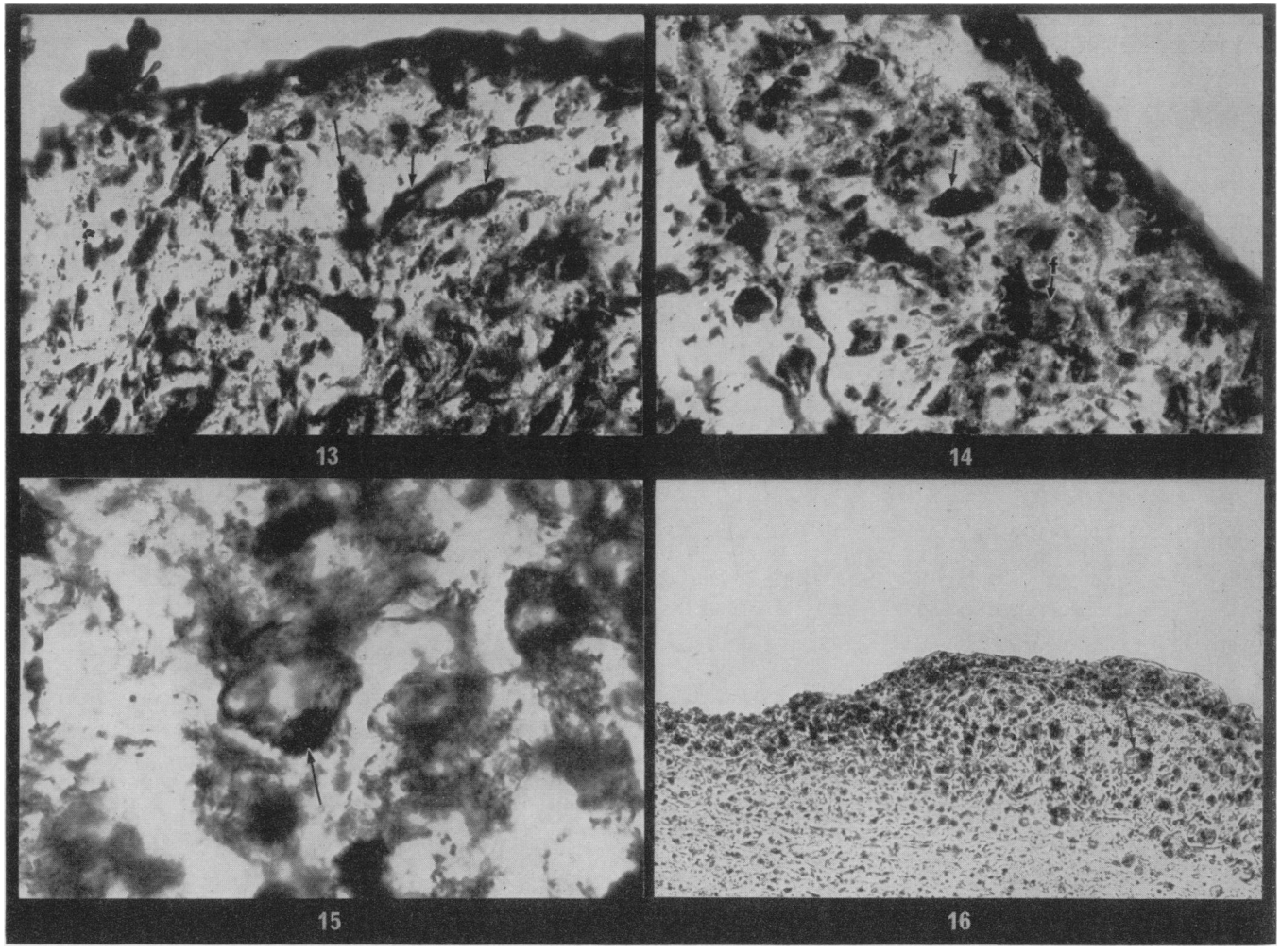

13 14

15 16

Cookson.

This is a very large cell, irregular in outline but generally round rather than elongated in section.

These cells do not have a surrounding basement membrane, nor do they have a fibrillar cytoplasm with associated densities. On the positive side, they show numerous peripheral cytoplasmic processes which are phagocytic tentacles and invariably, evidence of active phagocytosis can be found with accompanying breakdown and digestion of phagocytosed material.

The cytoplasm is extremely foamy being literally stuffed with vacuoles many of which are membrane bound and contain residual bodies, myelin figures and other debris. Cytoplasmic organelles tend to be obscured by the profuse accumulation of these vacuoles but small mitochondria, ribosomes, some rough surfaced endoplasmic reticulum and various types of lysosomes can be recognized. The cell nucleus has no particularly unusual features and a well developed nucleolus is common.

All the above features are illustrated in Fig. 7–12.

As mentioned previously degeneration and breakup of this second type of foam cell is frequent (Fig. 9, 10) and debris from these cells accumulates in the extracellular substance of the plaque.

Quite commonly, even with the electron microscope, evidence of mitotic division is seen at least involving the nucleus of these cells (Fig. 11). Most probably such division does only involve the nucleus for binucleate cells have been observed and the large size of these cells should be borne in mind.

The morphological characteristics of this second type of foam cell, particularly the evidence of rampant phagocytosis, indicate that it is a macrophage. Further confirmation of this view was obtained from the silver impregnation method of Marshall and the histochemical studies for acid phosphatases.

With Marshall's (1956) technique metalophil cells were found in the majority of the plaques examined. The most uniformly impregnated cells were confined to the superficial layers of the plaques. However, even in these, cytoplasmic vacuoles could usually be seen for these are active macrophages and cells with a smooth, totally impregnated cytoplasm typical of the resting macrophage were rare. In the majority of cells taking the stain, vacuoles predominate with the metalophil cytoplasm confined to a peripheral rim, an appearance to be expected in the light of the electron microscopy findings. Fig. 13–15 illustrate these points.

The findings from the histochemical studies using Burnstone's method for acid phosphatase are shown by Fig. 16. Acid phosphatase activity was found in all the plaques examined. The most intense reaction, which tends to obscure cell detail in thick frozen sections, was seen in the more superficial layers of the lesions. In deeper layers, related to the gruel core of the plaque, separated positively reacting cells could be seen. Many of these were obviously foam cells.

DISCUSSION

My studies have demonstrated that in these experimentally induced lesions two types of foam cells are found. The first of these I have called the smooth muscle foam cell for it is undoubtedly derived from the smooth muscle of the arterial wall, that is, from the cells of the tunica media. The second type is a macrophage of reticuloendothelial origin and hence, considering the normal cellular composition of the arterial wall, most probably derived from the blood monocytes. Some further discussion of these conclusions is warranted.

In developing the concept of the smooth muscle foam cell, Altschul (1950) claimed that active migration of smooth muscle cells from the arterial media was an early key event in the pathogenesis of the atherosclerotic plaque. Such migration was said to occur through breaks in the inner elastic laminae and to be stimulated by the accumulation of lipid within the overlying tunica intima. These deductions were based upon light microscopy observations. My own work suggests a somewhat different sequence of events. In agreement with most workers the earliest detectable lesion appears to be a widening of the subendothelial layer of the intima from the accumulation of extracellular lipid. At the same time intracellular lipid begins to appear in vacuoles in the innermost layers of the smooth muscle cells of the media. The inner elastic laminae are intact at this stage and whilst the smooth muscle cells remain attached to these laminae they do not develop the changes described in their ergastoplasm. The inner elastic laminae now begin to degenerate, split and breakup. As this happens, the attached smooth muscle cells are released into the base of the developing lesions. Such release appears to be a passive phenomenon rather than an active movement in response to stimulus as suggested by Altschul's hypothesis of migration. It is after their release from attachment to the elastic laminae that these cells spread into the lesion and rapidly develop the changes described in their endoplasmic reticulum and golgi apparatus. These changes suggest active cellular synthesis probably of protein material. Now it is generally accepted that the smooth muscle cells form and maintain the elastic laminae and collagen fibres of the normal arterial wall. Furthermore, new collagen and elastin formation are a characteristic feature of atherosclerotic lesions and such fibrils were constantly observed in my studies. Since the second type of foam cell is a macrophage it is an unescapable conclusion that this collagen and elastin formation is a function of the smooth muscle foam cell. In summary these cells are released into the lesion because of the degeneration of the elastic laminae to which they are attached. This loss of normal cell-fibre relationship seems to activate the cells to attempt the formation of new fibres and hence the restoration of their definitive resting state and the repair of the damaged arterial wall. The accumulation of lipid by these cells may also be a synthetic phenomenon since they do not show active phagocytosis and it is well known that lipid formation does occur in atherosclerotic plaques.

The second type of foam cell, the reticulo-endothelial macrophage, is an extremely active phagocyte after entry into the plaque. Because of this, early forms of this cell were rarely observed with the electron microscope and thin sections. These early stages were readily demonstrated in thick frozen sections by the Marshall silver method and this, together with the histochemical evidence, leaves little doubt as to the nature of the cells. As described, these macrophage foam cells rapidly enlarge and reach a considerable size. They then degenerate and breakup. The debris resulting is added to the pasty gruel which forms the central core of the well developed plaque, indeed such cell remnants form a considerable proportion of this core. This dead material is, like pus in an abscess, a hinderance to the possibilities of healing of the damaged structure, in this case the arterial wall.

A logical conclusion from this discussion is that factors which discourage the invasion of the atherosclerotic plaque by the second type of foam cell and those which assist the reparative activities of the first type of foam cell could be expected to aid in the healing of the lesions and the restoration of the functional capaci-

ties of the arterial wall. Such factors would include lowering of the serum cholesterol levels, correction of elevated blood pressure and possibly suppression of macrophage activity particularly blood monocyte activity. The first two procedures are standard clinical practice and their rationale is thus supported by these studies. The third is a theoretical possibility suggested by these studies and accessible to trial.

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