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Observations with the Scanning Electron Microscope on the Development of Cholesterol Aortic Atherosclerosis in the Guinea-Pig

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Summary. By means of scanning electron microscope "en face" examination of aortic intimal surface, two phases are recognizable in the development of experimental cholesterol atherosclerosis in the guinea-pig. During the first phase, after about two months of hypercholesterolic diet, no intimal plaques are to be found, and an amorphous substance (fibrin? — lipoproteins?) is deposited as a diffuse covering of wide areas of the aortic intimal surface along with erythrocytes and platelets. During this first phase, localized "flattenings" of the intimal folds are also evident.

It is only during the second phase (after about four months) that intimal plaques become recognizable, forming at the bottom of these scattered flattenings, while the diffuse amorphous deposit of the first phase begins to disappear.

Zusammenfassung. Mit Hilfe des Raster-Elektronen-Mikroskopes wurde die Oberfläche der Intima der Aorta von Meerschweinchen, welche mit cholesterinreicher Diät gefüttert worden waren, von der Fläche her betrachtet. Dabei hat sich gezeigt, daß 2 Stufen der Entwicklung einer Atheromatose unterschieden werden können. Die erste Stufe wird schon nach 2 Versuchsmonaten deutlich, zu einem Zeitpunkt also, zu dem im allgemeinen eigentliche Plaques noch nicht nachweisbar sind. Dabei findet sich eine schlierige Substanz, welche der Intime aufliegt, und die wahrscheinlich aus Lipoproteinen, möglicherweise aus Fibrin besteht und die Erythrocyten und Thrombocyten enthält. Diese erste Stufe wird auch durch beschränkte Intima-Plicae-Glättungen gekennzeichnet. In der zweiten Entwicklungsstufe entstehen echte Intimaplatten.

In earlier reports data concerning some *orthological* aspects of the *endothelial surface of the aorta, vena cava* and *endocardium* of the guinea-pig, as seen with the scanning electron microscope have been recorded (Weber *et al.*, 1970). This orthological picture is characterized by the presence of intimal folds, microvillous projections and crateriform lacunae on the endothelial surface and of intercellular bridges between endothelial cells. These features are modified when guinea-pigs are subjected to a *hypercholesterolic diet*: *after two months* the intimal folds clearly show flattenings in circumscribed areas. The endothelial surface of such flattened areas appears to be thinly covered by an amorphous substance, possibly fibrin or a lipoprotein complex, which sometimes looks smooth, oftener almost spongy: in such areas the underlying endothelial surface is no longer distinguishable. Erythrocytes and platelets are to be seen sparsely distributed, and in varying quantities, on this amorphous covering or, sometimes, incorporated in its thickness. Further, the endothelial surface appears to be covered by a smooth, amorphous substance not only in the flattened areas but also in wide areas of the otherwise

normal-looking intimal surface of the aorta of the hypercholesterolaemic guinea-pigs examined up to now (Weber *et al.*, 1970).

This paper records our observations with the scanning electron microscope of modifications of the aortic intimal surface in guinea-pigs after four months of hypercholesterolic diet. Forthcoming reports will relate to histochemical observations on the same subject.

Material and Methods

Five male guinea-pigs weighing 250–300 g were fed a standard guinea-pig diet with 0.3% added cholesterol (delta-5 Cholesten 3-ol Stock n^0 CH–USP Sigma), dissolved in ether. A second group of 5 guinea-pigs (control group) was fed the same standard diet without added cholesterol. After four months, all the guinea-pigs were killed by bleeding.

Small portions of aorta were fixed for two hours in 2.5% glutaraldehyde (0.2 M phosphate-buffered at pH 7.2), evaporated with platinum gold alloy after dehydration and observed with a scanning electron microscope (JSM 2) in the Institute of Zoology of the University of Siena.

Results

The aortic picture of hypercholesterolic guinea-pigs after four months of treatment is still characterized by the presence, on broad areas of the intima, of the amorphous substance that uniformly covered most of the intimal surface characteristic of the aortas examined in earlier studies. In some areas, however, this thin amorphous covering is no longer evident, while a granular material mixed with erythrocytes and platelets is seen to have been deposited here and there. Scattered circumscribed flattenings are also to be found, and in such areas the picture of the intimal folds is much less evident. At the fourth month, moreover, at the bottom of some of these flattened areas, small round intimal plaques now protrude slightly, projecting in relief above the flattened surface. Such small intimal plaques do not look superficially uniform but are irregularly riddled with small "holes".

Thus, it seems evident from our observations that the development of intimal plaques in the aorta of hypercholesterolaemic guinea-pigs is indeed preceded by the diffuse deposition of an amorphous substance over wide areas of the endothelial surface. The deposit is already to be seen after two months of this dietary regimen. Irregularly scattered flattenings of the intimal folds are also evident.

The deposition of this amorphous substance on the aortic intimal surface, as well as the presence of many scattered flattenings of the intimal folds, should be interpreted as a first phase in the development of experimental cholesterol atherosclerosis in the guinea-pig. This first phase, hitherto undescribed, has been demonstrable thanks to the possibility of "en face" examination of the aortic intimal surface by means of scanning electron microscopy.

Later, but only after four months of hypercholesterolic diet, true intimal plaques are finally to be seen at the bottom of the scattered flattenings of the intimal folds. By this time, the smooth amorphous substance that diffusely covers the aortic intimal surface during the first atherogenic phase is partially undergoing dissolution.

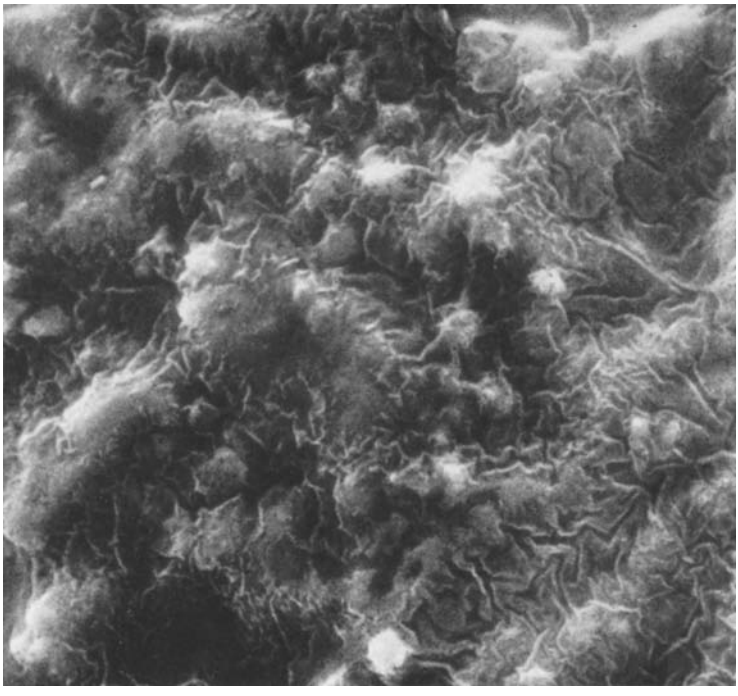

Fig. 1. Aortic intimal surface. Guinea-pig. After two months of hypercholesterolic diet. The endothelial surface is covered by a smooth, diffusely distributed, amorphous covering in the thickness of which erythrocytes and platelets are incorporated. × 1000

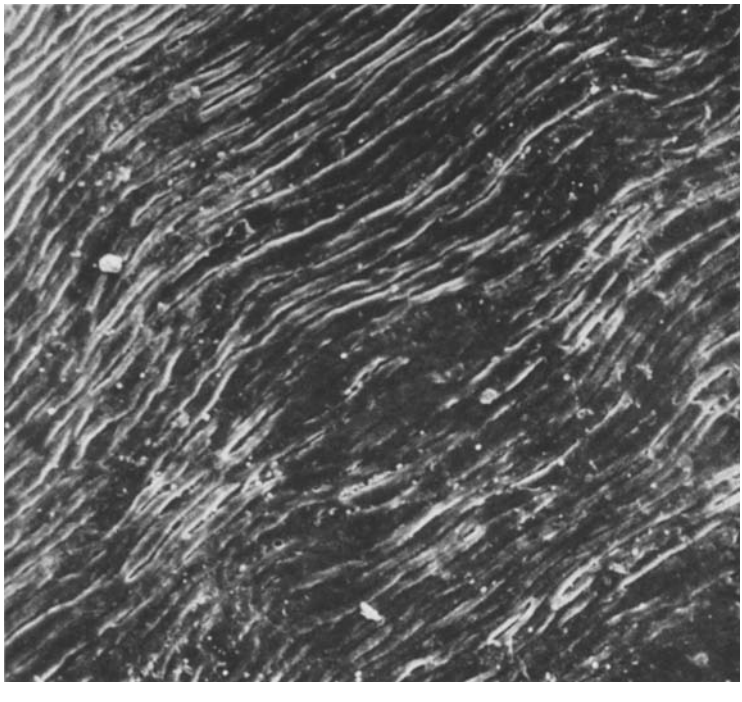

Fig. 2. Aortic intimal surface. Guinea-pig. After two months of hypercholesterolic diet. Scattered flattenings of intimal folds are evident. × 1000

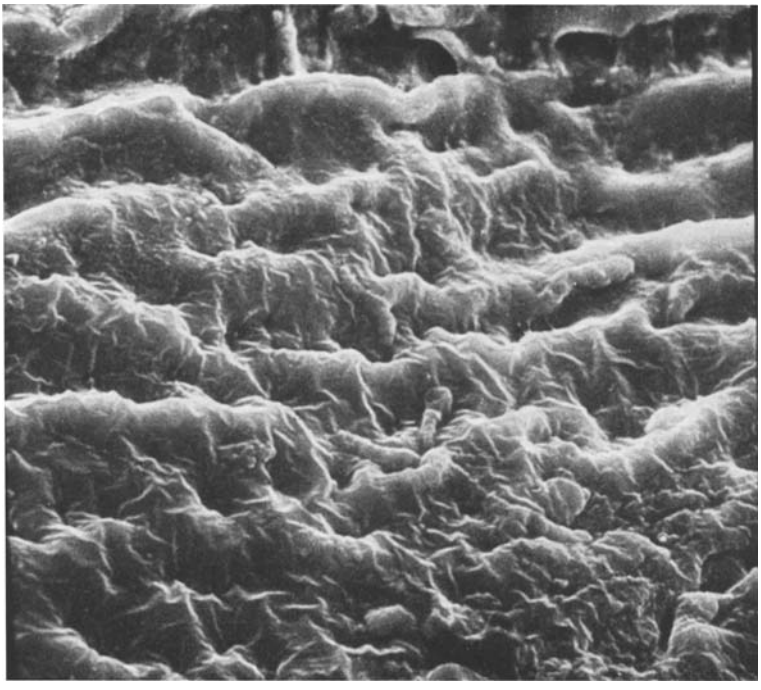

Fig. 3. Aortic intimal surface. Guinea-pig. After four months of hypercholesterolic diet the amorphous smooth covering is still evident in large areas. × 1000

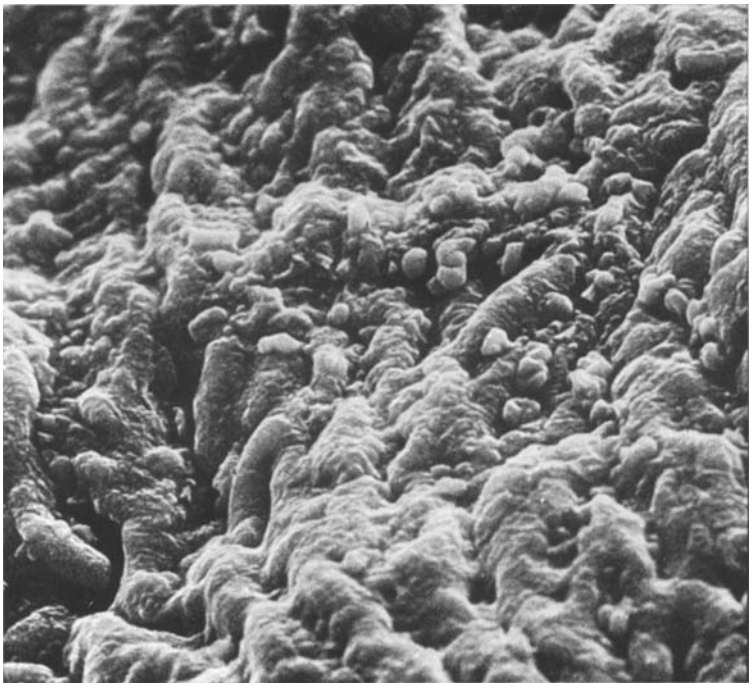

Fig. 4. Aortic intimal surface. Guinea-pig. After four months of hypercholesterolic diet. Erythrocytes and platelets are scattered on the amorphous covering or are incorporated in its thickness. × 1000

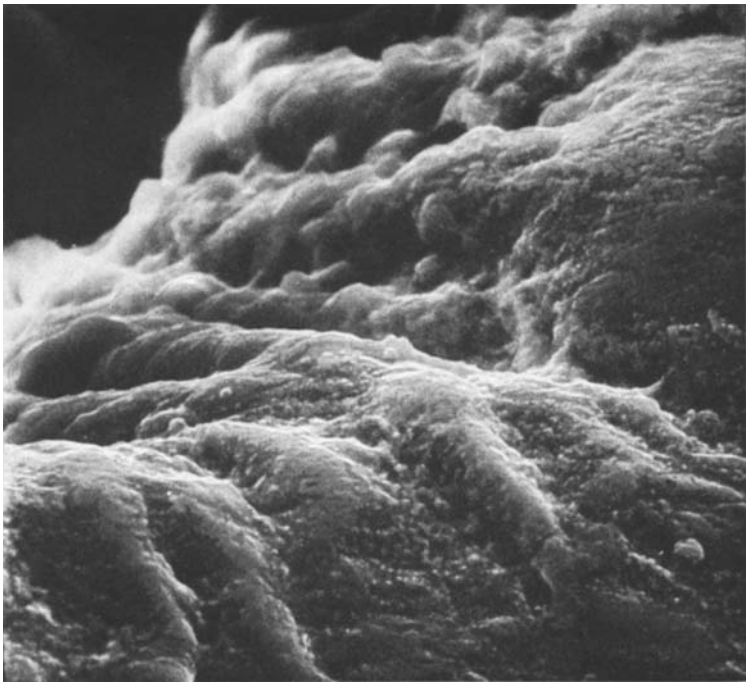

Fig. 5

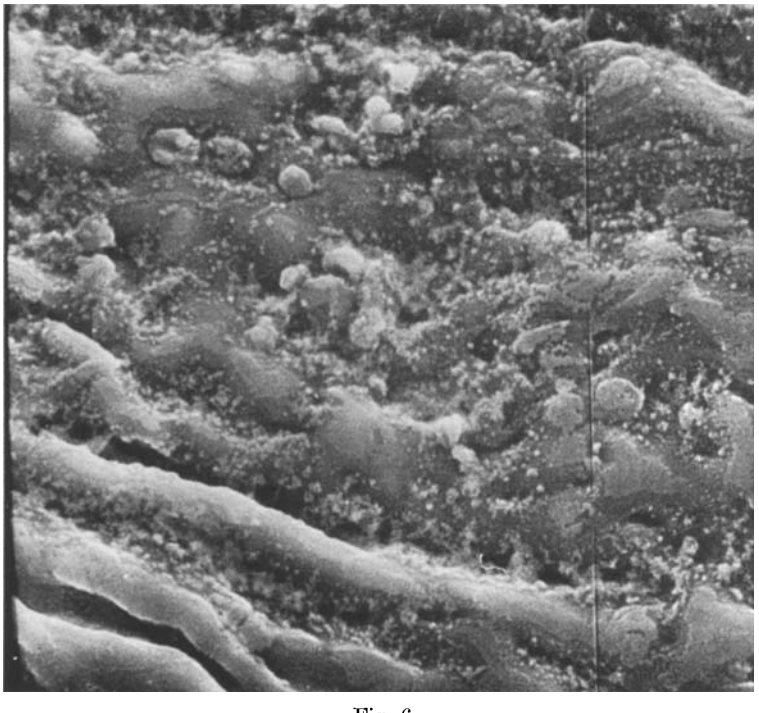

Fig. 6

Figs. 5 and 6. Aortic intimal surface. Guinea-pig. After four months of hypercholesterolic diet. A coarsely granular material is now to be found in areas near the origin of a collateral branch or at the bottom of flattened areas. $\times 100$, $\times 1000$

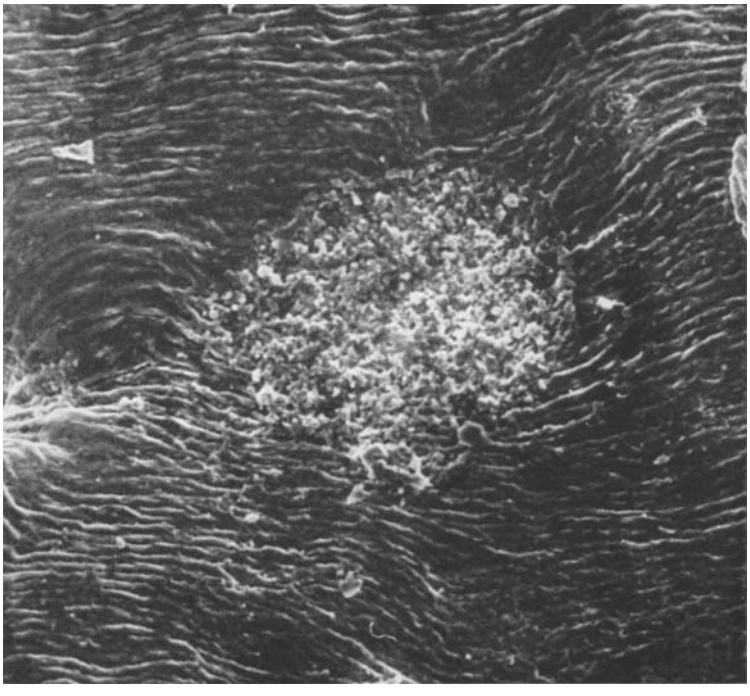

Fig. 7

Figs. 7–9. Aortic intimal surface. Guinea-pig. After four months of hypercholesterolic diet. Intimal plaques are to be seen at the bottom of flattened areas. × 100, × 300, × 700

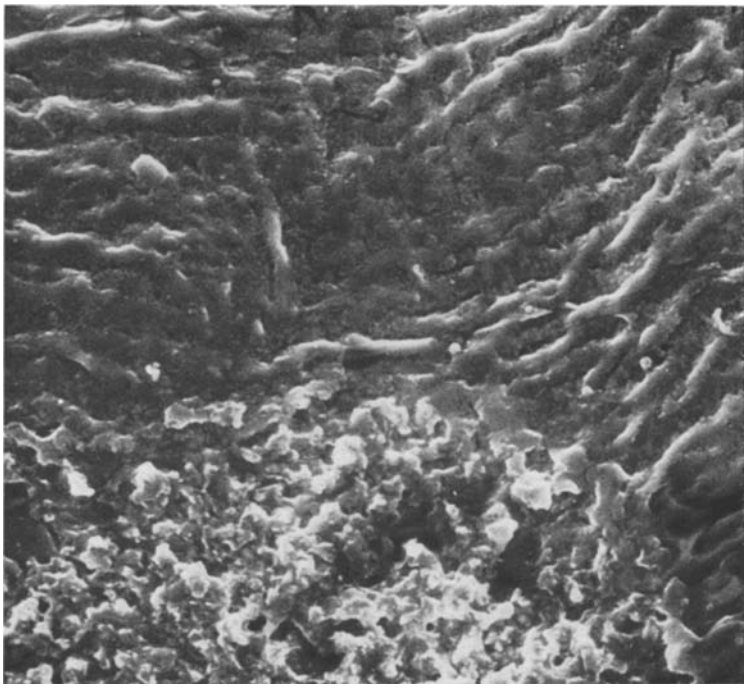

Fig. 8

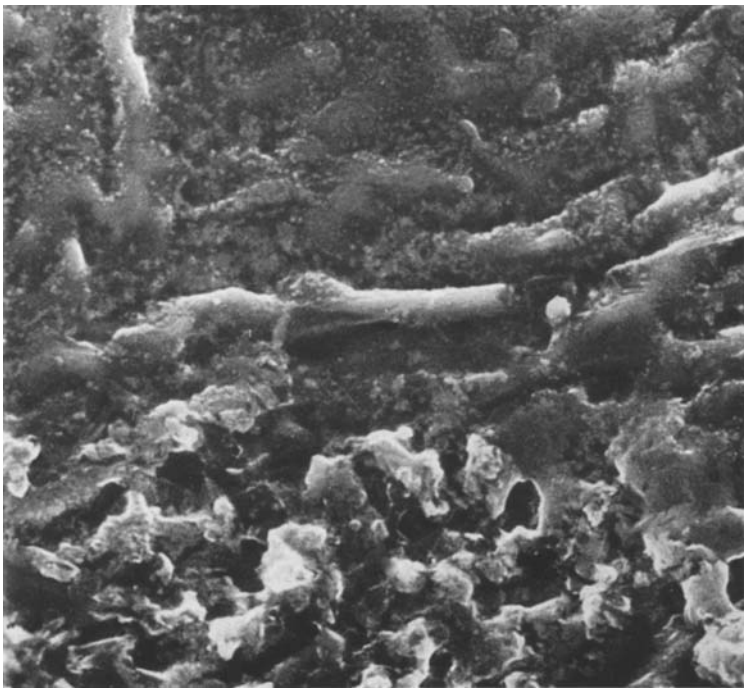

Fig. 9

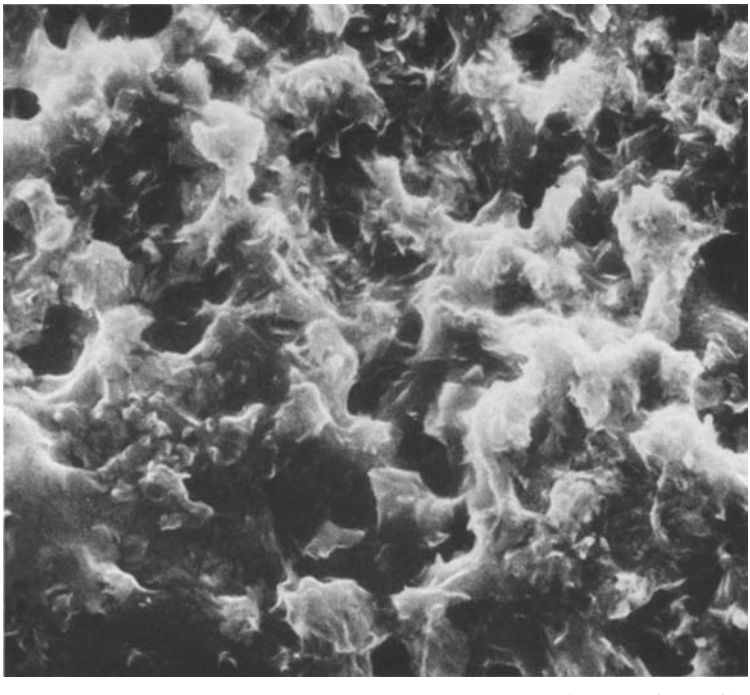

Fig. 10. Aortic intimal surface. Guinea-pig. After four months of hypercholesterolic diet. The surface of an intimal plaque. ×1 000

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