ATHEROSCLEROSIS AND ITS ORIGIN

Atherosclerosis and Its Origin

edited by

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Preface

With ever-increasing rapidity the information and literature concerning atherosclerosis is growing. It is impossible at the present time for any one individual to read and assimilate all the available information, especially that derived from disciplines far removed from his own. It would take at once an individual who was a clinician, pathologist, electron microscopist, histochemist, biochemist, and lipid chemist to even stand a chance. This volume is intended to serve as a starting point for the beginner in the field of atherosclerosis research enabling him to ascertain the state of this field at the present time. At the same time, the experienced worker in this field may find the information contained useful in helping him to fit information which he has in with that available from other disciplines. Although not intended for practicing physicians or medical students, they too may be able to see clearer the areas of controversy, especially with regard to diet and its effects on atherosclerosis as well as information as to the diagnostic tools available.

We have attempted to cover the field of atherosclerosis focusing on the human lesion, reverting to information from lower species when the information has not yet been obtained in man. We have included chapters on the histology, pathology, and metabolism of arterial tissue. Believing as do the more sophisticated workers in the field today that there is no one cause of atherosclerosis, we have included chapters on the role of ground substance, hemodynamics, serum lipids, hormones, diet, and the arterial wall metabolism in the development of the lesion.

In view of the importance placed on lipid metabolism in the development of atherosclerosis we felt the need of a chapter on the interrelationship of lipids in blood and tissues, since, for the most part, all that we measure at the present time is blood lipids especially in the clinical situation. Moving on then to experimental atherosclerosis, we felt the need for a description of the naturally occurring lesions in animals, for in order to be certain that the experimental results are due to the manipulation of the experimentor one must be extremely aware of what occurs spontaneously.

We then find discussed methods of inducing the lesion which are in vogue today, followed by a description of the state of metabolism of the aortic wall in the rabbit and then the histochemistry of the lesion in the rat, dog, and man.

We must express our appreciation to all authors for their cooperation and care in preparing this manuscript. The Editors have felt that in a

field such as this it was not their place to suggest the removal of a concept because it does not agree with those in vogue today or, in fact, with other authors in this same volume. We feel that any concept which is provoking will generate ideas and will therefore generate further knowledge.

Finally a word of thanks to the staff of Academic Press for their cooperation and care in helping to bring this work to press.

September, 1963

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G. H. BOURNE

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I. Introduction

For a number of reasons, the morphological features of normal arteries cannot be fully described in a single chapter. Consideration must be given to species differences, to differences from one site to another in one species, and to certain changes in these features which occur with the passage of time. Time is a particularly important variable in

an analysis of the structure of human arteries, since an understanding of aging effects provides the basis for an intelligent approach to the study of arteriosclerosis.

In this chapter I have tried to survey certain recent work which I consider to be especially significant and, in particular, I have emphasized the electron microscopic studies. Electron microscopy has contributed greatly to the understanding of arterial structure, and although many problems remain, it is true to say that even the most casual observation of properly prepared arteries with the electron microscope provides answers to at least a few of the problems on which investigators of the past spent many futile years. Only part of this gain has been brought about through a higher resolution provided by the electron microscope; much has depended upon better preservation and embedding techniques. Recent improvements in technique, particularly in such electron staining methods as the phosphotungstic acid stain used by Pease and Molinari (1960), have contributed substantially, and further development of such methods will continue to do so.

Since the subject of this book is arteriosclerosis it may not be impertinent to emphasize the importance of regarding the arterial wall as a structure containing living cells having, in greater or lesser degree, such general properties of protoplasm as growth and metabolic activity. The changes observed in arteriosclerosis are, of course, dependent upon many factors, but not the least of these are the living processes of the cells in the vessel wall. It is therefore necessary, in reviewing the morphology and histogenesis of arteries, to include some description of the dynamic properties and potentialities of their component cells. For this reason I have discussed the results of various experimental procedures designed to investigate these properties.

II. Endothelium

A. The Height of Endothelial Cells

The endothelium is a cellular layer which forms a continuous lining throughout the arterial tree. The lining is extremely delicate, and unless gently handled and properly fixed it will be lost or distorted. A good way to fix it in large blood vessels is by perfusion *in situ* under slight pressure for a short time, and follow this by immersion fixation. In smaller arteries of experimental animals the endothelium is fixed well by dribbling fixative for a few minutes over the adventitia while blood continues to flow, and completing fixation by immersion. The vessels should not be cut into small blocks until partially dehydrated.

The height of arterial endothelium may often seem to depend upon the size or type of artery. In small arteries or arterioles the cells are often cuboidal or even columnar, while in the aorta they are usually squamous. However, if the vascular system is perfused with the fixative under slight pressure the endothelium of smaller arteries also appears flattened. Obviously, since the smaller vessels undergo relatively greater post-mortem contraction the height of the endothelium is simply an index of the relative amount of contraction. There is probably no fundamental difference in the height of endothelium in different kinds of arteries.

The high parts of the endothelium of contracted small arteries contain the nuclei which often appear to bulge into the lumen because they come to lie on the "crests" of the folds in the internal elastic lamina (Altschul, 1957). The cytoplasm between the high points may often remain extremely thin. With the electron microscope such thin areas can be observed to represent only the combined thickness of the inner and outer plasma membranes and a fraction of a micron of interposed cytoplasm.

B. Cell Boundaries

The endothelial cells have a long axis paralleling that of the vessel. They are fitted together in a mosaic pattern best appreciated by a study of the surface with whole mounts or with split-off endothelium (Häutchen preparations). Various techniques may be used to make such preparations but probably the simplest is to stain the fresh tissue with silver nitrate. Details of this method are given by Lautch *et al.* (1953) who were largely responsible for reviving the method and applying it to the study of arteriosclerosis. An excellent review of the subject is included in a paper by Poole *et al.* (1958), in which they also describe their modifications of the technique by which the most exquisite rendering of the cell boundaries is accomplished. Sinapius (1956) has also provided a comprehensive review of the literature on the staining of vessels with silver, and from this and his own observations he concluded that chloride ion is responsible for the binding of silver. Chloride may be leached out by soaking in distilled water, but if the tissue is subsequently treated with chloride ion, the endothelium regains its original staining affinity. After studying the distribution of the silver lines in relation to the nuclei, Sinapius (1956) concluded that they did not always represent the intercellular boundaries. Often the nuclei appeared to be located immediately under silver lines. The correctness of this conclusion has been denied by Florey *et al.* (1959).

There are changes in the pattern of silver lines which are related to aging. Cotton and Wartman (1961) studying the aorta, pulmonary artery, and renal artery of man, found that the principal changes were some loss of polarity of the cells (so that the pattern was no longer orderly in relation to the vessel axis) and the appearance of greater

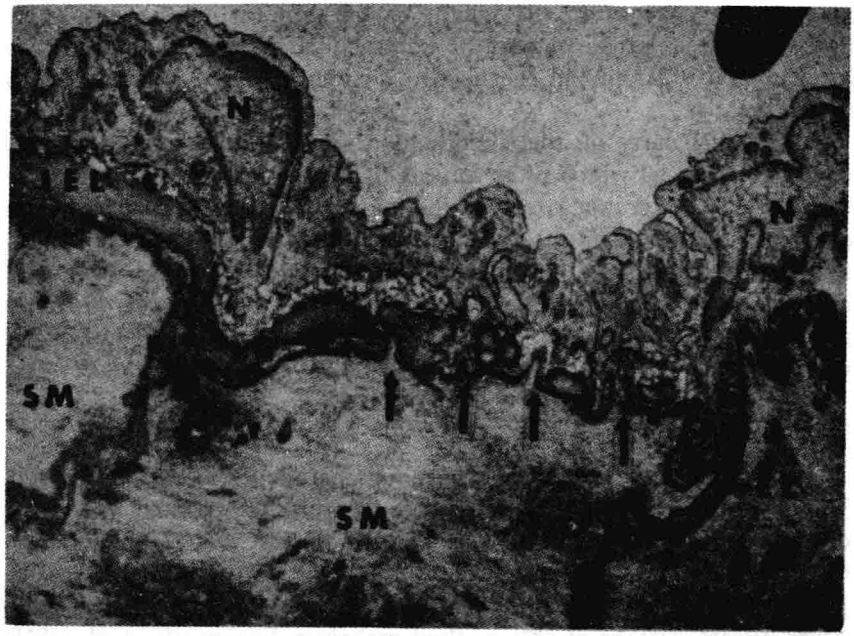

FIG. 1. Low power view of the inner surface of the constricted rat femoral artery showing high endothelium and bulging nuclei (N). The long dark lines in the endothelium indicate points of junction between adjoining cells. The endothelium is separated from the internal elastic lamina by a light space containing the endothelial cell basement membrane and some collagen fibrils. The internal elastic lamina (IEL), which is continuous with elastic lamellae (EL) separating the smooth muscle cells (SM) of the media, shows several fenestrations into which the smooth muscle basement membrane, or sarcolemma, projects (arrows). Vestopal section, stained with phosphotungstic acid. Magnification: × 9700.

variation in the size of cells, with some many times the normal size. In addition, very large cells having up to 9 nuclei in a rosette arrangement were also observed in old age.

With the electron microscope the abutting plasma membranes (Figs. 1 and 2) of adjoining endothelial cells are readily seen. The plasma membrane of one cell is separated from that of its neighbors by a remarkably uniform "gap" of about 100 A. In fact, there is probably no

real "gap" at all, for the area of low density may be filled with a part of the membrane not visualized in the electron micrograph. The perfectly parallel course of the two membranes, in spite of convolutions, would suggest that this is the case. The extent and complexity of this interface, as seen in sections cut at right angles to the plane of the endothelium, depends largely upon the heights of the cells. In the high endothelium of small vessels fixed by immersion a long, wavy, often interdigitating boundary exists; in the lower endothelium of perfusion-fixed vessels the interface is usually shorter and straighter. The longer interface is associated with vascular constriction and the shorter one with dilatation. The implication of these observations is that, in some cases at least, the morphological basis of the boundary between the cells need represent nothing more specialized than simply their coming into contact with each other, for its extent can be varied by varying the degree of constriction of the vessel.

On the other hand, in some vessels endothelial cell junctions show special morphological features which suggest a function in holding the cells together. In the rabbit coronary artery certain stretches of plasma membrane along surfaces of mutual contact are occasionally seen to be slightly more dense than other parts (Parker, 1959). True desmosomes are rarely seen in arterial endothelium although they are present in certain types of capillaries (Farquhar, 1961).

Fine intracellular fibrils, sometimes seen in endothelial cells of small vessels may perhaps be associated with desmosomes. Florey and Grant (1961) and Rhodin (1962) stated that fibrils were commonly seen. Fibrils observed in certain endothelial cells of the human dermis and subcutaneous tissue were considered by Hibbs *et al.* (1958) to represent contractile elements. They believed that special blood vessels possessing such contractile endothelium might be important in the regulation of skin temperature by their control over blood flow. In view of the common occurrence of fibrils in endothelial cells (Fig. 2) the existence of special contractile endothelium is questionable. The function of fibrils in endothelium, and in such cells as those of the hepatic parenchyma, is still not understood.

C. Intercellular "Cement"

The question of the existence of intercellular "cement" at endothelial cell junctions has been under study for many years. A review of the evidence for supposing that a cement material exists between endothelial cells and is stained by silver nitrate was given by Chambers and Zweifach (1947) and Zweifach (1959). According to McGovern (1955) the cement