CORONARY ARTERY INTIMAL HYPERPLASIA

by

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ABSTRACT

Intimal hyperplasia in the proximal portion of the right coronary artery was investigated by using longitudinal sections of the artery, to determine the amount of intimal and medial thickness, and the amount of deviation from the normal of the internal elastic lamina. One hundred and one samples in the 0-30 year age range were used and the results correlated with other known information about the individuals.

Findings:

1. Intimal thickness increases directly as age increases.
2. There was a sex difference in the 16-30 year age group (males greater than females), but not under 15 years.
3. Intimal thickness increases significantly as the amount of internal elastic lamina change increases.
4. Medial thickness increases significantly with increasing age.
5. Intimal thickness increases significantly as medial thickness, heart mass and body mass increase, and increases at a more rapid rate than any of the three.
6. The amount of elastic lamina change increases significantly with age only between 1 and 30 years.

These findings are correlated with the possible factors in the etiology of intimal hyperplasia.

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INTRODUCTION AND OBJECTIVE

In 1951, Duff and McMillan (39) expressed the view that the fact that arteriosclerosis has its inception in childhood and is almost invariably present after adolescence, at least in some degree, indicates an inherent susceptibility of the human species to arteriosclerosis developing under apparently normal conditions. The structure of the intima undergoes considerable change from infancy to adult age and it is the purpose of this thesis to investigate the growth of the intimal layer of the coronary artery with the aim to determine if it is a product of some postnatal factor, such as injury, or if it is a part of normal physiological growth.

The origin and evolution of the arteriosclerotic process are obscure and doubts can be entertained about all features of arteriosclerosis as to their significance in terms of cause and effect, but, the early structural alterations of such a chronic disease as arteriosclerosis are important for an evaluation of the histogenesis and possible pathogenesis of the process. In 1915, Klotz (81) issued a dictum on such studies: "A study of the earliest stages of the lesions in the tissues of man is still the most secure upon which to base conclusions". This statement actually was a criticism of works who made faulty conclusions regarding arteriosclerosis, based on an examination only of late lesions.

The intimal layer of the coronary arteries has been found to be relatively thicker than that of other vessels with areas

of thickening present even in infancy, and which become more prominent with increasing age. These lesions show a certain parallelism to the gross findings in adults. (40)

The significance of microscopic findings in the intima are considered by many to be uncertain as we still do not know why the process occurs, why certain individuals get more clinical disease than others, or why the process tends to involve certain parts of the arterial tree. However, hyperplasia of the intima is generally regarded as an early non-lipid phase of atherogenesis and therefore detailed knowledge of the coronary artery throughout life may open new lines of research which may enable the developement of measures to prevent or at least delay the end stages, even though some investigators (117) still claim that at the present it seems very unlikely that we shall discover a cure for arteriosclerosis or coronary artery disease, since some part of the process is irreversible.

A progressive investigation of coronary sclerosis using clinical material is impossible due to the inaccessibility of the coronaries, therefore studies must use animal experiments and post-mortem material. To date, animal experiments are not adequate to study the early changes because they do not exactly parallel the human lesions.

To investigate the possible causes of intimal hyperplasia, we studied post-mortem samples from 101 random autopsies in the age range 0 - 30 years by making longitudinal sections of the proximal portion of the right coronary artery. The findings from these sections were correlated with other known information about the individuals.

Review of Literature on Intimal Hyperplasia

Definition of intimal hyperplasia

The subendothelial layer, or intima, is thought to be the precursor site of later arterial disease, the etiology of which is unknown, but inferences have been drawn from studies based chiefly on microscopic structural changes. It is a fibro-elastic thickening lying between the endothelium and internal elastic lamella (104). The intima is fundamentally an area of proliferation of various components consisting of fibroblast-like cells which are probably responsible for the deposition of a complex mixture of collagen bundles, acid mucopolysaccharides, proliferating and degenerating elastic fibres, and smooth muscle cells (21). The various intimal components are all the elements needed for the construction of a tissue which resembles that of the subjacent vascular wall. It is suggestive of a reparative tissue aiming at the formation of a new blood vessel wall (115) which in all probability starts as a compensatory mechanism in response to some injury (62).

The Normal Coronary Artery Wall

Before discussing in detail the intimal layer of the coronary artery it is necessary first to define what is to be accepted as the normal. That is, if the intimal hyperplasia is to be considered abnormal then descriptions must procede from a baseline - the normal wall of the coronary artery.

One of the earliest descriptions of a so-called normal tunica intima was by Rindfleisch, 1872, (127) "The tunica intim a exhibits striated lamellae of Henle which are finely striated,

wavy layers of basis-substance (of connective tissue), in whose interstices flattened, lenticular corpuscles are imbedded. In these cell-containing spaces, the opposed surfaces of the basis-substance exhibits a peculiar homogeneous lustre, morever, they present a double contour, which makes each cell appear as if surrounded by a special capsule. The cell-containing spaces appear of a stellate form, with branching prolongations which anastomose with one another." From his description, he is referring to a subendothelial layer with the various components mentioned earlier already present. Even as recently as 1965, (52, 55, 86) some investigators regard the normal intima as a thickened layer with the lining endothelium, a layer of sub-endothelial connective tissue, in which there may be a few muscle fibres, and an internal elastic membrane. These workers agree that the intima undergoes progressive growth from an exceedingly thin structure at birth but consider this still the normal intima. (55) A more accepted conception of the normal arterial wall is the picture before any hyperplasia (1, 29, 40, 49, 61, 109, 117, 119), consisting of a single layer of the endothelial cells with only a few strands of collagen between it and an intact internal elastic membrane. (41, 97). Under the Electron microscope, (119) the intima is seen to consist mainly of endothelial cells with junctions resulting from overlapping of the cell margins which abut on the internal elastic lamina. The endothelial cells send filiform processes to the media through the fenestrations, possibly for nutrition of the media through the relatively

impermeable elastic membrane by the many caveolae intracellulares and small vesicles of their cytoplasm. The normal internal elastic lamella is seen to be a continuous fenestrated subendothelial tube of homogeneous refractile material of moderate density, in which numerous fibres of 500A diameter with no periodicity are imbedded (106). It has an irregular surface and sends branches to the media between the smooth muscle layers (119). The longitudinal corrugations are due to post mortem muscular contraction (41).

Osborn (1963) (117) claimed that in 465 infants dying at birth the coronary artery demonstrated this normal appearance. Others (40, 61, 63, 88, 91, 103, 130, 131) have demonstrated deviations from the normal in premature infants (i.e. prenatally), and in newborns.

Hyperplasia of the coronary artery intima

Intimal growth is largely a post natal process and continues, in many cases, through life and varies from individual to individual.

Rokitansky, 1852, (136) was one of the first to describe the growth of the intima and regarded it as a precursor of other conditions including the atheromatous process. He described it as "an excessive formation and deposition of the lining membrane of the artery derived from the mass of the blood, and at the same time constitutes hypertrophy of this membrane and, being split into lamellae, is drawn away in the form of strata, varying in thickness". He found it varied from a thin soft moist and succulent nature in the translucent or early lesions, to a thicker, dense, dry, tough and elastic nature in the older or opaque portions. He noted that it varied in extent from local patches to the whole

vessel, especially prominent at bifurcation of a vessel, and regarded the whole process as related to a chronic inflammation. Virchow, 1856, (11, 56, 87, 128) also found that the earliest changes in arteriosclerosis occurred in the intima and consisted of a gelatinous swelling in this layer with accumulation of material resembling mucus and delicate elastic fibres in this "structureless diaphanous intercellular substance". Concomitant with this alteration he noted an enlargement and multiplication of the connective tissue cells to form localized thickening. This is a similar picture to that which Rindfleisch described as a "normal intima".

Since Virchow's and Rokitansky's description of the intima the views have been changed and refined with the developement of the newer techniques. Intimal growth is very similar to the reparative process of connective tissue following injury, with a relatively orderly sequence of the various processes resulting in the complex picture of the hypertrophied intima. (67, 106)

The first demonstrable deviation from the normal wall is alteration of the internal elastic lamina which undergoes fraying, splitting, fragmentation and reduplication (29, 34, 40, 63, 88, 103, 105, 139, 148). There is a slight disagreement as to the earliest changes. There is a question whether perhaps the initial change is characterized by several simultaneous alterations including the elastic changes, an increased production of acid mucopolysaccharides at the site of the elastic changes, and the appearance of fibroblastic cells in the subendothelial area (55, 106, 107, 110, 117, 142). There is general agreement, however, that these changes are all present early in the process (29,40,41,49,

51,88, 117, 125, 139,148).

Following the initial change the processes can be divided into the following components: (107)

1) proliferation of fibroblastic cells
2) deposition of increasing amounts of acid mucopolysaccharides
3) further degeneration of elastic lamina
4) regeneration of new elastic tissue
5) formation of collagen fibres
6) hyaline degeneration of connective tissue occurring late and proceeding to further degenerative lesions.

In early childhood the process begins focally but eventually become diffuse in the sense that most of the intimal surface is involved, some areas to a greater degree than others (35).

The foci seen in childhood are of a cellular nature exhibiting the elastic lamina changes, increased mucopolysaccharides, and the intrusion of some muscle cells between the gaps of the internal elastic lamina. These cells are associated with the production of delicate elastic fibres and collagen (29, 34, 131). The muscle cells reorient themselves to form a layer of longitudinal smooth muscle inside the remnants of the internal elastic lamina (34, 41, 64, 99, 105, 134). This has been called the musculo-elastic layer (39, 40, 64, 93, 105). With advancing age there is a progressive increase in the above components with a relative increase in new elastic tissue and more and more intercellular connective tissue elements, mainly collagen (41, 64, 88, 93, 99, 105). With further progression there is complete dissolution of the remnants of the internal elastic lamina and degeneration often of some of the new elastic tissue resulting in an indistinct

division between media and intima, with hyalinization of the connective tissue elements and appearance of lipid as droplets, or foam cells and eventually as cholesterol clefts (34,35,40, 105).

In the past the different stages in this continuous process have been segregated. First the "musculo elastic layer" was mentioned. As the elastic components increased mainly inside the longitudinal muscle layer an "elastic hyperplastic layer" was defined, and with the deposition of large amounts of collagen especially on the inner surface "the connective tissue layer" was defined (29, 40,41, 47, 64, 103, 112). Since these changes are phases of continuous tissue alteration and since the layers depend upon the stage of the lesion when seen, it is unnecessary to continue to define these layers. Also, since the changes are usually uneven in an artery, all these layers can occur at one time (40, 64). The media under the internal elastic lamina may also exhibit a reorientation of bundles of smooth muscle cells in a longitudinal direction parallel to those in the intima, thus presenting a difficulty in defining media from the new tissue or intima (47, 131).

Until recently there had been confusion as to the type and origin of the cellular components of the intima. Originally the cells of the intima were thought to arise from the circulating blood (87) and later they were thought to be fibroblasts (106, 128) from their appearance and also from the fact that being almost the only cell present in the intima, they had to be responsible for the production of new elastic fibres, collagen and mucopolysaccharides. In other words, they were held responsible for

producing the extracellular material of repair, (4, 21, 105,106, 128, 146, 155) and for phagocytosis (29, 34).

With electron microscopy these cells have been shown to be in intimate association with and to produce these extra cellular structures (27, 53, 56). With light microscopy these cells also possessed the characteristics of smooth muscle cells including the shape of the cell and nucleus with its coarse chromatin distribution, the eosinophilic staining qualities of the cytoplasm, and the presence of numerous fibrils parallel to the long axis of the cell. These fibrils, of which the coarse ones were situated at the cell boundaries and the more delicate ones in the cytoplasm proper, were seen to extend beyond the cell boundaries to adjacent cells, stained intensely red with Masson's trichrome and gave a positive reaction with PTAH and therefore were considered to be myofibrils (56, 69, 112). The cells also have been claimed to possess the capability of contractility (69). All these characteristics suggest that these are smooth muscle cells and not fibroblasts. (4, 99).

With the electron microscope more evidence has been obtained to confirm their identity as smooth muscle cells. Myofilaments similar to those of ordinary smooth muscle, that is, of 50-100 A^{o} diameter were seen massed together and confined to a thin layer close to the plasma membrane and other cytoplasmic organelles including apparatus for protein synthesis, were more widely scattered in the central cytoplasm (27, 28, 53, 56).

There have been various theories as to the origin of these smooth muscle cells including dedifferentiation from endothelial cells regenerating following damage (8, 69,)

or differentiation from fibroblasts (29). Another theory is that the smooth muscle cells arise directly by metaplasia of pre-existing primitive totipotential mesenchymal cells in the area (21, 35, 76, 148). A more widely accepted concept is that discontinuities in the internal elastic lamina allow the active migration of smooth muscle cells from the media by growth and amoeboid action. With light and electron microscopy smooth muscle cells of the media have demonstrated orientation with processes extending through gaps in the internal elastic lamina into the intima (8, 34, 53, 54, 112, 131). With the electron microscope mast cells, lymphocytes, monocytes and histiocytes have been seen, indicating possible penetration from the lumen, but these are not claimed to give rise to smooth muscle cells or extracellular elements (53, 56, 112).

We still do not know why the smooth muscle cell, rather than the fibroblast, appears to form the intima, and what influences the smooth muscle cell to produce elastic fibres, collagen and acid mucopolysaccharides. It does fullfill a pathophysiological requirement by rendering the area "elastic" by virtue of its contractility. With all the preceding evidence, McMillan (96) stated that he is still not convinced that it is possible to tell if these cells are less differentiated mesenchymal cells that are assuming a differentiation resembling that of smooth muscle, or whether they are a pathological alteration in the normal cyto-differentiation of smooth muscle cells.

Internal Elastic Lamina change

As previously mentioned, changes from the normal intact

elastic lamina which is a smooth, longitudinally corrugated, continuous, homogeneous subendothelial tube with fenestrations, have been seen as early as 36 weeks of gestation. These changes consist of localized stretching, fraying, and splitting, with rupture or degeneration, forming discontinuities in the lamina. The elastic lamina is often seen to be split, an appearance which either represents formation of new elastic fibres by the smooth muscle cells or a true splitting of the original elastic lamina. Eventually this destruction or reorientation of elastic tissue results in a complete disappearance of the original normal internal elastic lamina (17, 29, 34, 88, 99, 105, 108, 109, 117, 142, 150).

Simultaneous with the above process is the production of a network of new delicate wavy elastic fibrils arranged in layers between the circularly arranged smooth muscle bundles (29, 53, 99, 108). Depending on the association of these new fibres with large amounts of collagen or smooth muscle tissue they formerly were referred to as the elastic-hyperplastic or musculo-elastic layers (40, 41, 108). This new elastic tissue has been shown to be different from the internal elastic lamina and therefore not simply degenerating or calcifying old elastic elements of the lamina, by the demonstration of altered staining properties of the new fibres (21). If this were a physiological effort to produce a new internal elastic lamina, identical staining reactions might be expected. A gradual transition from the earliest new fibres to the later lesions has been demonstrated (59, 88).

Under the light microscope the earliest changes were

accompanied by a swelling of the hyalin core of the internal elastic lamina with an appearance of bead-like material coating the lamina (59, 105). Under the electron microscope these beads have been identified as new fragments of elastin (53). The first lesions consisting of swelling and decreased density of the internal elastic lamina have been interpreted as lipoidal degeneration or lipids entering into elastic protein to form a solid solution of lipoprotein in and around elastic tissue. (3,6, 120, 148, 105). There is some evidence to support the idea that lipid imbition represents the primary insult to the arterial wall (44), but it may also be that lipid is deposited in the wall because its transfer is blocked by the abnormal intima.

Appearing later (nine years of age) is a progressive deposition of calcium either on the surface or within the substance of the elastic elements which has been considered as a manifestation of aging and possibly the source of calcium in the later degenerative lesions (4, 21, 84, 104, 114).

The function of the internal elastic lamina is to passively prevent over-stretching of the arterial wall by maintaining tension against the distending force of blood pressure. The medial smooth muscle, by active contraction, also contributes to the maintenance of tension but requires a continuous expenditure of energy and so is less efficient. The peculiar elastic behaviour of a blood vessel has been shown to result mainly from the combination of elastin and collagen fibres in the wall. Slight stretching of the wall will put stress on the elastin fibres but the much less extensible collagen fibres do not reach their unstretched

length until the vessel is considerably distended. Therefore the elastic tissue is the main control against dilation. According to Hooke's Law, as the wall is stretched it resists, not proportionally to each stretch, but more and more strongly at each additional stretch (31, 88).

There have been several suggestions as to the cause of degeneration of the internal elastic lamina including enzymatic reactions and other humoral injurious agents (21), and actual mechanical splitting by the migrating smooth muscle cells (132). The most widely accepted theory is that of mechanical injury due to stretching by a rising intra arterial pressure, or the prolonged maintenance of normal pressures which are greater than the tensile strength of the elastic tissue (110). Whatever the cause of the changes in the internal elastic lamina, there is a resultant loss in elasticity and decrease in the tensile strength of the wall, and this is followed by an attempt to reconstruct new elastic tissue to compensate (21, 76, 88, 105, 110). It has been suggested and qualitatively shown that the amount of intimal growth is associated with the number of breaks or changes in the elastic lamina, indicating that both are part of a reparative process (7, 63, 91, 129, 131, 147). This is also supported by the fact that electron microscopy has shown the smooth muscle cells to beproducing the new elastin.

Mucopolysaccharide Changes

The accumulation of small amounts of acid-mucopolysaccharide is recognized as one of the earliest changes in the subendothelial area (21, 59, 108, 110, 125, 142, 150, 161).

In the normal intima, the internal elastic lamina is encased in a pool of mucopolysaccharide (124, 163). Alterations in the acid mucopolysaccharide are thought to constitute a major feature in the pathogenesis of arteriosclerosis as they are constant and prominent features (16, 106, 128, 150). It has been shown that acid mucopolysaccharides are constantly associated with degenerating areas of elastic tissue as seen by increase in concentration in areas of fraying and splitting of the internal elastic lamina. They are also seen surrounding all new elastic tissue formation (16, 29, 105, 112, 128, 150, 163). It was thought that this ground substance was the result of the degenerating elastin (21, 111, 150), an imbition of an imperfect, possibly depolymerized ground substance, or partially derived from mast cells (128). However, it is probably an extracellular secretory product of the smooth muscle cells (16, 106, 128, 155) and it is formed as a precursor for incorporation into collagen and elastic fibres (26, 105, 150). This is similar to any regenerative process in the body after some injury (34, 59, 140). The acid mucopolysaccharides have been shown to increase quantitatively with age, up to about 50 years, in association with the growth of the intima (9, 17, 29, 84, 110, 163). No statistically significant study has been found to verify these quantitative statements. Along with the quantitative age changes there is a qualitative change in the ground substance. The infant intima contains hyaluronic acid as the major component of the ground substance. By histochemical studies the following changes have been found to occur with increasing age: (16,17,32, 155, 163).

1. There is a relative and absolute increase in sulfonated acid mucopolysaccharides and acid mucopolysaccharides bound with proteins, mainly chondroitin sulfate A and C (especially after age 20 years) along with chondroitin sulfate B, heparitin sulfate, alpha-heparin, and kerato sulfate.
2. There is an increase in neutral mucopolysaccharides and sialic acids.

These same variations were found in the extracellular as well as intracellular areas indicating the area of production.

In addition to the role of mucopolysaccharides in the formation of fibres, the change in the type of ground substances may contribute to an alteration of the permeability of the subendothelial tissues which may facilitate the trapping of subtances in the intima, or influence the local metabolism of lipids (26, 32, 58, 128, 150, 155).

The changes in mucopolysaccharide metabolism may reflect a change to an abnormal activity of the intimal fibroblastic cells to produce excess sulfonated acid mucopolysaccharides. In certain inherited disorders of connective tissue (Hurler's Syndrome or Gargoylism) there is a severe disturbance in mucopolysaccharide metabolism. This is demonstrated by an excess secretion of chondroitin sulfate B and heparitin sulfate. The results of this defect are reflected in all areas of the body including the arterial intima which shows tremendous hyperplasia with mucopolysaccharide deposition, especially in the coronary arteries (94, 100).

As has already been described, there is an increasing amount of collagen deposition in the intima with age. This is a reflection of a reinforcement mechanism to strengthen the arterial wall at the sites of internal elastic lamina destruction. The collagen consists of fine and coarse collagen fibres. The young intima consists chiefly of fine collagen which is easily soluble by collagenase. The ratio of coarse to fine fibres increases with age and severity of elastic change (163). Collagen fibres have been seen binding together ruptured ends of the internal elastic lamina (163) and elastic fibres are surrounded by a compact network of reticular fibres. The collagen fibres are presumably synthesized by the smooth muscle cells perhaps utilizing the carbohydrate macromolecules as a template or pattern on which the protein molecules are oriented to form the collagen.

The following protein changes occur with age (155):

1. There is an increase in tyrosine, cysteine, reticulin, fuchsinophilic procollagen and elastin.
2. There is an increase of bi-refractive scleroproteins.

In the first two decades the collagen present in the intima exists mainly as a weakly polymerized solution of particulate macromolecules, because the predominant substance present is hyaluronic acid, which does not form permanent bonds with proteins, and whose molecule, because of its large size, prevents fibrillary accumulation and precipitation of polypeptide chains in collagen solutions. After the second decade, the production of sulfonated acid mucopolysaccharides, which are small molecules, is unable to prevent the accumulation and precipitation of polypeptide chains into collagen

solutions. Stable bonds between the proteins and acid mucopolysaccharides form fibrils and fibres. Fine easily digestible collagen is chiefly associated with chondroitin sulfates A and C, whereas older coarse fibres are most frequently connected with chondroitin sulfate B. The ratio of chondroitin sulfate B to chondroitin sulfate A and C increases with age (17, 155, 163).

The medial changes consist of a constant increase in thickness with general body and heart growth, with the greatest increase in number of elastic fibres and smooth muscle cells occurring in the first two decades (61). Starting in the second decade, the coronary artery media has decreased elasticity with clear cut calcific changes of the elastic tissue, along with fraying, fragmentation and regeneration of the fibres, similar to the process in the intima (84). These new and degenerating elastic components seem to have an affinity for calcium. There is still a constant total elastin content after the second decade indicating that the loss of elasticity is probably due to the clacification rather than the loss of elastic tissue. It is postulated that these changes may establish a substrate for later lipid changes, but also they are claimed to be an independent process unrelated to atherosclerosis and probably a coincidental association with it, in the coronary artery (29, 85, 104). If one accepts the theory that mechanical injury stimulates intimal hyperplasia, then a loss of elasticity of the media would make the weakened arterial wall more susceptible to the forces of intravascular pressure. However, this loss of elasticity due to calcification has not been demonstrated in infants when the intima is already thickening. Calcification may contribute to later

proliferation (17, 24). Changes in the internal elastic lamina are accompanied by important effects on the smooth muscle of the media. Large numbers of the cells near the internal elastic lamina turn inwards forming an inner radial coat, and are then seen to penetrate into the subendothelium where they continue to proliferate (117,133).

Localization of lesions

It is generally agreed that the intima of the coronary artery has a greater relative growth than that of any other vessel in the body (61, 131, 137, 138). Before there is generalized intimal thickening, only segments of the coronary artery are involved (159). It may well be that local anatomical factors determine the sites of lesions, as the affected areas in infants are usually the same sites as in adult arteriosclerosis (55, 88, 103). There is, however, not a complete uniformity in similar situations in the individual coronary or at the same sites in different individuals. Hyperplasia of the intima is most pronounced in the proximal portions of the left and right coronary vessels (53, 61, 101,158). In infancy as well as later in life, the epicardial portions of the coronary vessels have been found to have a much thicker intima than the mural portions, or the parts invested by myocardium, suggesting some mechanical influence (37, 38, 50, 57). This helps to support mechanical theories of etiology especially those involving intravascular pressure and shearing stress. It has also been noted that there is an increase in intimal thickness at the site of branches or bifurcation of the coronary vessels suggesting a hemodynamic effect (48, 61, 127, 152). This is linked with the foetal intimal

muscular cushions which have been observed at mouths of branches of the coronary arteries as well as other muscular arteries (36, 132, 157). These cushions of longitudinal muscle and elastic tissue have been interpreted by some as a means to regulate the blood flow to the branch (38, 139, 157) but generally are considered to be a reaction to hemodynamic stress; i.e., a buffer zone or a remodelling for wall reinforcement, which is in continuity with, or will develop into the picture of intimal hyperplasia which has been described (29, 34, 131, 132, 133).

In the right coronary artery of the dog the distribution of maximal hyperplasia was similar to that of the human. The changes were in the proximal two centimeters on either side of the sino-atrial and conus artery origins and continuing into the two branch arteries, and at the ventricular branch at the margo acutus (25, 61).

It is claimed by many investigators that intimal thickening occurs more frequently and earlier in the left coronary artery, particularly in the anterior descending branch, than in the right coronary (40, 47, 61, 101). This is thought to be partly due to the fact that the left coronary artery is subject to more tensions than the right during the cardiac cycle (85, 110). Others claim that any difference in intimal hyperplasia in the three major vessels, (left anterior descending, left circumflex, and right coronary) are not statistically significant (13, 72, 91, 111). However, whether there is a quantitative difference between left and right coronaries or not, there is general agreement that the pattern of development of intimal hyperplasia is similar in both,

leading to the conclusion that the same process operates in the two arteries (64, 98, 158).

Definition of terms

Arteriosclerosis means, literally, "hardening of the arteries" and was used first by Lobstien in 1829 (14, 121) to describe the thickening and hardening of the walls of the arteries. It is a term used to describe a complex mixture of degenerative and reparative processes which lead to increased rigidity, diminished elasticity, and often decreased calibre of arteries, commonly accentuated at focal points, but with a tendency to spread and become confluent (21, 22, 23, 65). It is the result of many types of etiology and pathogenesis. It includes disorders of large and small arteries affecting the intima, such as atherosclerosis, elastosis, regenerative intimal thickening, and the acute form of Raynaud's disease, and disorders affecting the media, such as medial hypertrophy or atrophy, Monkeberg's disease, cystic and fatty degeneration. Therefore arteriosclerosis encompasses the reaction of the total arterial wall as represented by its components.

Atherosclerosis is a type of arteriosclerosis and therefore a more specific term (96). It was used first by Marchand in 1904 (121) to describe the process of the primary fatty and atheromatous degeneration of the arterial intima (14). A W.H.O. technical report (210) described atherosclerosis as "a variable combination of changes of the intima of arteries consisting of the focal accumulation of lipids, complex carbohydrates, blood and blood products, fibrous tissue and calcium deposits, and associated with medial change." (19). It is a nutritional-metabolic disease

of multifactorial causation (46, 78, 145).

Intimal hyperplasia is therefore an early indication of arteriosclerosis as any forms of this disease which involve intimal lesions must occur in this thickened intima. To define arteriosclerosis one must include primary contributors to its morphology and pathogenesis such as the intimal hyperplasia, changes in the internal elastic lamina and medial changes (109). Jores went so far as to claim that any splitting of the internal elastic lamina is a manifestation of arteriosclerosis (95). Even authors who consider the intimal growth a "normal" physiological response recognize that there is a transition to a pathological process consistent with arteriosclerosis (101, 110, 139).

The intimal layer of the arterial wall has been considered to be a pre-atheromatous proliferation (35, 46, 62, 108, 159) but since atherosclerosis has a complicated pathogenesis the association is still not completely clear (29, 56, 66, 103). Intimal fibrous thickening of arteries is a progressive process, probably age-related (1, 4). It is necessary to establish a distinct cause and effect relationship between these changes and the atherosclerotic process, as fatty streaks have been seen without intimal thickening. It has been demonstrated that there is a direct relationship between the frequency and severity of atheroma and the extent of the subendothelial growth (40, 41, 49, 61, 112). Geiringer (57) claims that there is a critical depth of the coronary intima of 0.35 mm. after which the chances of atheroma development are greatly increased. The earliest recognizable lesion of atherosclerosis is claimed by many to be the early lipid seen in the

hypertrophied intima (4, 35, 55, 109). The decreased pliability and elasticity of the thickened intima may promote a change in metabolism of intimal cells which encourages lipid deposition (41). There is a continuous sequential change from childhood to adult life which suggests that the early micro-alterations in the subendothelium precede, and probably lay the ground work for the lipid deposition, hyalinization, and calcification of atheromatous plaques (63). Even authors who claim that intimal hyperplasia is not essential for atherosclerosis admit that its presence seems to favour lipid deposition (148).

Accepting the concept that atherosclerosis has its beginning in infancy, a study to elucidate its etiology and pathogenesis must focus on the incubation period of the disease rather than on its advanced manifestations and complications. The process must be followed from its inception in the perinatal period through childhood and adolescence into adult life in order to reconstruct the histogenesis of late, clinically important lesions. The focal point for all the changes in atherosclerosis is the arterial wall. It therefore must be studied from all angles.

Past correlations of intimal hyperplasia with various factors.

A. Age and media:

The qualitative relationship of intimal hyperplasia with age has been discussed. There have been numerous descriptions of the progressive changes in the intima with increasing age but few quantitative studies. The first evidence of such a study was by Mann in 1912 (97) who measured the intima of the aorta in microns at different ages and found a linear relationship with age:

Age	Intima	Media
Newborn	6	650
16 years	54	856
35 years	124	996

Only three specimens were used to establish this relationship in the newborn to the 4th decade period! Since then, five authors have produced some quantitative evidence showing a progressive intimal increase with age. Moon et. al. in1952 (107) showed a linear relationship of intima versus age in the range 0-30 years but failed to state the number of specimens used or how they were evaluated. Groom et. al. in 1964 (63) showed a similar relationship in 177 specimens but used average percentage encroachment on the lumen as a criterion. Neufeld et. al. in 1962 (116) showed a progressive increase with age of the ratio of the intima to the total vessel thickness using 100 hearts but failed to produce evidence to show a statistically significant relationship; as his graphs do not show one. Levene in 1956 (89) used 98 hearts to show an age-intima relationship but his graph also raises doubts as to the significance of the intimal growth in the 0-20 age groups. Lober in 1953 (91), using 173 specimens in the 0-30 year range, measured the thickness of the intima as a percentage thickness of the arterial wall and related the averages to age producing a very convincing graph, but still failed to verify it statistically. Although some of the previous workers have mentioned using the proximal parts of the coronary arteries with the larger amounts of hyperplasia, none other than Moon et. al. (107) mentioned or used multiple sections along the vessel.

Most have related the intimal thickness to the media (137) or to the total arterial wall (37, 91, 116) before relating it to age, rather than working out a direct relationship.

Since histological features are non-uniform in their distribution throughout the coronary arterial tree the element of chance can be minimized only by numerous measurements either by serial cross sections or by multiple longitudinal sections. Also, quantitative judgements of small gradations of intimal hyperplasia are difficult technically. Any errors in measurements can be minimized by the examination of all specimens by one observer.

Ignoring criticisms, these earlier studies have shown a progressive increase of the intima from birth to the 4th decade and beyond. There is controversy as to the period of maximum rate of intimal growth. Lober (91) claims the sharpest rise is in the 20-30 year group, but Groom (100) and Moon (105) state that the most rapid rate of intimal growth occurs for several months following birth and then slows.

There is evidence to show that the thickness of the media progressively increases with age but the intimal thickness increases relatively more rapidly than the media and there is therefore a rise of relative intimal thickness with age (89, 91, 116). There is also evidence that the mean external diameter of the coronary artery increases with age (116).

B. Internal Elastic Lamina:

The progressive age changes of fraying, fragmentation and production of intimal elastic tissue have been described. Many authors have remarked on the close association of the amount of

elastic change with the growth of the intima and suggested a causal relationship between the two events (131). However, few have attempted to quantitate elastic change. Moon et. al, 1952, (107) produced a graph showing the elastic degeneration versus age but failed to say how he arrived at the results. Groom et al, 1964, (63) graphed the qualitative presence of fraying or fragmentation of the internal elastic membrane which too, showed a progressive increase with age. He also failed to give details as to his methods. Lober, 1953, (91) graded the amount of elastic lamina change from 0 - 12 years, and plotted this against age producing a straight line increase in destruction with age. None of these experiments have been verified statistically.

From the preceding results we conclude that there have been indirect inferences concerning the relationship of internal elastic lamina change and intimal thickness, but no direct investigational work has been found on this subject.

C. Sex:

Although some studies have failed to substantiate a difference between male and female in the thickness of the intima in the infant coronary artery (142), most investigators have shown that males have a thicker intima than females from birth onwards (38, 48, 61, 90, 133) suggesting a possible hormonal factor. Dock, 1946, (37) examined the coronary arteries of 12 neonatal infants and showed a statistically significant difference in the amount of intimal hyperplasia between males and females. Fangman and Hellwig, 1947, (51) also used 12 infants to draw the same conclusions. Their studies have been critisized for the small number of specimens,

(112, 139). Minkowski, 1947, (103) demonstrated a sex difference in infants older than 24 hours which is supported with statistical evidence by Lober, 1953 (91) who found a significant difference over the age of one month, using 173 specimens in the age group 0 - 30. After the age of 10, the progressive rate of intimal growth was shown to be the same in both sexes even beyond the menopause. This suggests that the hormonal effects may not be fundamental. Using 400 specimens from age 20 - 80, Avtandilov, 1963, (13) showed that the sex difference persisted until age 60, after which there was no difference.

The only study relating internal elastic lamina degeneration to age was done by Lober, 1953, (91) who demonstrated an increase in elastic lamina changes in males compared to females at all ages but states that the only group statistically significant is the 40 - 49 year group.

In the literature we reviewed, we found no studies relating intimal hyperplasia or elastic change to body weight, heart weight, or blood pressure even though hypertension appears to increase the incidence of clinical coronary disease. It is generally acknowledged that diabetes tends to cause clinical coronary disease to develop at an earlier age than in a non-diabetic person (91). Also, there are inferences to suggest that there is an increase in intimal thickness in individuals with many forms of chronic disease, but there are no studies to substantiate these theories.

There are reported studies showing a difference in intimal hyperplasia in different races (49, 133). But in each case it is a comparison of an American white population with a race in some

other part of the world or of a completely different socio-economic background. Supporting this Groom et. al. (63) used genetically similar populations from different countries and showed a significant difference in the amount of intimal thickening, internal elastic lamina changes, and gross atheromatous lesions.

THEORIES OF ETIOLOGY OF INTIMAL HYPERPLASIA

Although much has been written on the subject of the etiology of intimal hyperplasia, it is still poorly understood and there is very little definite knowledge about the etiology. It is essential to determine why and how certain changes take place and to understand the mode of the development. This might aid us in directing an intelligent approach to prevention and treatment of arterial disease.

Since the changes of intimal hyperplasia are part of a process which predisposes to, or causes, eventual dysfunction they are, by definition, considered to be pathologic. These changes progress with age but are more than a simple process of aging, as different sites in different arteries manifest changes in different degrees at different times. The actual causes of intimal proliferation are unknown but are considered to be a combination of many factors, both local, (e.g. altered metabolism and permeability in the vessel wall) and systemic influences, (e.g. endogenous or exogenous factors in the blood, hemodynamic forces, genetic and environmental influences, and intercurrent disease). (46, 50, 61, 62, 66, 73, 106, 111, 142, 144)

It is generally agreed that the early intimal hyperplasia of the coronary arteries represents a phenomenon of adaptation to high demands made on these vessels in the first months of life, which continues into later life.

An early explanation of the process of intimal hyperplasia was given by Virchow (1856) (56) who believed it to be a

primary inflammatory overgrowth of the intimal connective tissue resulting from mechanical irritation of the arterial wall by the pressure of the blood at critical points. This overgrowth of the intima would interfere with the nutrition of the cells. Rokitansky (1852) (136) held an opposing view that the stratified hypertrophy of the intima was the result of an excessive deposition of a fibrinous substance derived from the blood on the lining. Thoma (1911, (153) believed that the intimal hyperplasia was due to irritation of the wall, not only by mechanical factors, but also by a toxic substance in the blood, probably a metabolic product.(82)

Since the 19th century, numerous theories have been proposed for the etiology of intimal hyperplasia, but basically all of them are variants of the three above mentioned theories. The most widely accepted concept is that intimal proliferation is similar or identical to a connective tissue reparative process following injury (23, 59, 62, 106). Whether a continuous stress is present, or multiple recurring small episodes, the arterial wall attempts to reform a structurally sound wall (147), i.e. the various components of the arterial wall attempt to adjust to changes in local environment (21). Any type of insult to the arterial wall interferes with oxygenation and nutrition of the vascular wall, leading to degenerative and proliferative changes. This is probably the fundamental causal mechanism regardless of etiological agent (73). The etiological agents can be summarized in four groups:

1. physiologic and pathologic mechanical trauma.
2. metabolic effects including physiochemical disturbances of the blood, of endocrine, vitamin, or nutritive origin.

3. inflammatory agents

4. physiologic aging processes

Regardless of the specific physiologic alteration in the wall, the histologic reaction to injury is always the same, namely a proliferation of the intimal connective tissue (50, 105).

Assuming an injury is the initiating event in the process of intimal hyperplasia (34, 125) which is manifested by the early changes in the internal elastic lamina and intercellular substance (102, 114), what are the forces that produce the injury? Since intimal hyperplasia is not diffuse, but is localized in certain arteries, and even certain sites within these arteries, these forces must be localized, at least partially (46, 50, 152). In the literature innumerable factors have been implicated as injurious agents to the arterial wall, the general classes of which are summarized above. Only a few of the most important ones will be discussed below.

PHYSIOLOGIC AND MECHANICAL TRAUMA.

Although numerous factors are involved, many authors agree with Virchow's view that mechanical injury is a major initiating cause.

The mechanical theory of the genesis of intimal hyperplasia is that the changes are the end result of general and local hemodynamic factors acting over a long period of time, and a a result of this wear and tear by such forces the artery undergoes a reparative process, an an adaptation or compensation, to maintain a relative constancy of the mechanics of blood flow. That is, intimal hyperplasia is a sequel to fluid dynamics applied to the vascular

system which is modified by other secondary factors such as age, sex, nutrition, lipid metabolism, hormones, drugs, associated diseases, and other causes (20, 109, 151). But it is basically the mechanical factors which are contributory (4, 112).

As has been stated, the initial event is probably the internal elastic lamina change which can result from intra-arterial tensions greater than the tensile strength of the elastic tissue (107, 135) and lack of support from a possible relative medial weakness (33, 88, 131, 149).

The degree of intimal hyperplasia has been postulated to vary with the amount of hydraulic tension, augmented by other hemodynamic factors (22, 143). The other hemodynamic factors which are contributory are intravascular pressure, cardiac thrust, vibration, volume flow, gravitational forces, viscosity and friction, and shearing forces (22).

The coronary arteries, because of their position as branches of the first part of the aorta, are subject to exceptional hemodynamic forces. They are subject to unusually high pressures because while they are pumped full of blood under high pressure they are also subject to pressure from outside due to myocardial contraction. This wide range of pressure which occurs in the coronary arteries, because of their proximity to a high pressure system, (accentuated by the elastic recoil of the aorta) and their proximity to the ventricles, must have some bearing on the severity of intimal hyperplasia in them (4,5, 32, 40, 50, 52, 85). They also have a fixed origin and are fixed where they branch or enter the myocardium (20). Between these areas there is considerable

movement where there is little outside support.

Supporting a mechanical causation is the fact that the areas of maximal intimal hyperplasia usually are found at areas of altered hemodynamic forces. These areas are where the arteries taper, curve, bifurcate, and at the mouths of branches (20, 40, 55, 85, 115, 117, 127, 131, 132, 151, 152). It is at these sites that the maximum effect of pulsation and turbulence from changes in directional flow are felt. From this pulsation turbulence and changing lengths during the cardiac cycle, shearing stresses result at branchings and angulations in the lines of least resistance and of possible cleavage(at the internal elastic lamina-media junction, due to their different stretch capacity). This can cause injury and initiate a reparative process (2, 6, 7, 41, 133). Viscus drag of the blood also helps to produce a longitudinal movement of the inner part of the wall, therefore contributing to the shearing stress (92).

Also at these sites of curves is the postulated hemodynamic suction pressure and lifting action of blood flow on the intima. This is due to the negative pressure engendered by the conversion of potential energy to kinetic energy in a flowing liquid when it traverses a constriction in a tube (Bernouilli's Theorem). The suction and lifting action contribute to eddying and turbulence (31, 36, 45, 82, 92, 151).

It has been found that in the epicardial or proximal portion of the coronary arteries there is much greater intimal hyperplasia. The wall of the epicardial coronary artery may be thicker by 33% than that of the mural part (139). In this area,

unprotected by the myocardium, the hemodynamic forces from over-distention, changing lengths, and angulations are greatest. This is the only difference from the mural portion of the same artery (57, 108). Following on this, since males generally have slower pulses and higher stroke volumes than females, and therefore greater stresses to this part of the coronary artery, males should have a thicker intima (37).

With an increase in blood pressure all the hemodynamic forces would be increased and therefore increases of intimal thickness would be expected. This has been confirmed (110, 158, 159).

Supporting the mechanical injury concept is experimental evidence in animals. Following acute mechanical injury to the arterial wall there is a degeneration followed by a reparative process histologically similar to intimal hyperplasia in humans (67, 79, 118, 140, 142, 152).

Intimal hyperplasia has all the earmarks of a compensatory process or adaptation to a progressively rising intra-arterial pressure or the prolonged maintenance of normal pressures (30, 110). This implies that intimal hyperplasia is inevitable and proressive throughout life (109). Blood pressure varies with age and is closely related to height and weight. Although fetal intra-arterial pressures have not been recorded, there is significant increase at the time of birth. Significant increases of intra-arterial pressure also occur during the adolescent period, and many variations occur at this time before the more stable levels of adult life. Exercise, excitement, coughing and

straining may raise the systolic pressure of children as much as 40-50 mm. Hg. above their usual levels (164). All these changes and fluctuations in intra-arterial pressure may contribute to injury of the vessel wall. However, the detailed distribution of the lesions cannot be always explained on a hemodynamic basis as the distribution is not always uniform in similar sites in different individuals, therefore other factors must be operating also (55). Also, since hydraulic stresses are common to all, other factors must be responsible, at least in some part, to explain tremendous variation of intimal hyperplasia in individuals.

METABOLIC INJURY

Several investigators have claimed that lipid appears by imbibition from the plasma in the cells and intercellular substance of the endothelium and subendothelium before changes occur in the internal elastic lamina (8, 113, 154), and that this represents the initial process of intimal hyperplasia (51, 86). The intimal fibrosis, in other words, represents a reactive process to the lipid.(110). It is true that lipid in the arterial wall will stimulate intimal hyperplasia (120), but it is usually considered an adjunct to the initial process as large studies have revealed that while some early lesions contain lipid, in the majority of cases the lipid appears appreciably later than the fibrous hyperplasia (107, 117). It is unusual to find lipid in lesions in individuals under five years of age.

In some diseases resulting from endocrine disturbances, such as diabetes, there is an increase of intimal hyperplasia in

younger individuals, implying that the change in the hormonal content of the body may injure or more probably promote faster growth of the intima (91). Reisman (126) correlated the maximal intimal hyperplasia and infiltration in the male in the 10 - 20 years period with the maximal androgen production. However, during this adolescent period many other changes take place including increased caloric, protein, and vitamin A intake. Baló (14) postulates that the elastic fibers are regulated by pancreatic elastase which is, in turn, checked by elastase inhibitors and suggests that an alteration in this balance may be contributory. He also suggests that acid-base balance alterations will injure elastic fibers which therefore would implicate many disease processes.

Early metabolic changes found in the arterial wall both in the amount and types of acid mucopolysaccharides, proteins, and lipids are reflections of changes initiated by some other process, but they contribute to changing local metabolism and permeability of the wall (46, 124, 155). Therefore they are important in the pathogenesis of intimal hyperplasia.

There is much experimental evidence to implicate lipids in the atherogenic process, but little to suggest that they are the initial insult to the arterial wall. It has been shown experimentally that lipids are capable of injuring the arterial wall with a subsequent proliferative reaction mimicking intimal hyperplasia (14, 34, 85, 147, 149). It is agreed that lipid can contribute in the stimulus of fibrous hyperplasia, but, for it to be the initial insult would require that lipid be seen early in

the process. This has not yet been confirmed.

INFLAMMATORY AGENTS

There have been claims in the past that intimal hyperplasia is an inflammatory process (11, 127). However, other than localized inflammations such as syphilitic aortitis, inflammatory agents from an infective organism have no bearing on the arteriosclerotic process (23). Experimentally, numerous agents, both organic and inorganic toxins, have been used to injure the arterial wall and promote a reaction which will simulate intimal hyperplasia (34). Most of these have been used in combination with lipemia to promote an atherogenic process.

AGING PROCESS

Intimal hyperplasia has been considered as a part of a normal growth process or an expression of the constant remodelling of the arterial wall as it increases in size (53). While the constituents of the wall change in their composition with age biologically similar to many other body tissues, aging is insufficient to explain the tremendous variation in different people with age and in different parts of the arterial tree (8, 73, 84, 146). Therefore intimal hyperplasia should not be regarded as an inevitable by-product of senescence alone but as a result of the many factors which are operating as an individual ages(77). Many writers have postulated a genetic factor influencing the degree and distribution of intimal hyperplasia, a theory which is very difficult to evaluate and prove (38, 50, 52, 108, 123). For example, Nahum (114) suggested that some people are born with elastic tissue better able to withstand the years of stretching

and stress.

THROMBOGENIC THEORY

There are still investigators who agree basically with the etiology proposed by Rokitansky (3, 43, 136). That is, that intimal hyperplasia is the end product of mural fibrin deposits on the intimal surface, resulting in a pathological process consisting of intimal proliferation and thickening. And these lesions are composed of several layers, suggesting recurrent formation of fibrin deposits, each followed by fibroblastic proliferation and organization by endothelial cells and cells from the arterial wall replacing the thrombus with fibrous tissue (2, 12, 34, 43, 44, 45, 68, 70, 89, 122, 145). This would help to explain the decrease in lumen size with intimal hyperplasia, whereas injury should promote a dilation (44, 45, 55). Also,in older lesions a continuity between the fibrous and thrombotic lesions has been seen (45). However, these transition stages have not been verified in the very early lesions (161). It is difficult to conceive that these early changes result from encrustation or imbibition of fibrin into the intima. On the other hand it appears quite reasonable to suspect that localized areas of tissue injury may be very susceptible to fibrin deposition and eventual replacement by fibrous tissue, resulting, after a long period, in a fibrous plaque. Therefore the thrombogenic process could account for a part of the intimal hyperplasia but certainly not all (4, 42, 44, 45).

Still, the genesis of primary concentric intimal hyperplasia is obscure. There is no fundamental histological differ-

ence between the tissue which can result from organization of fibrin thrombi and that resulting from the proliferation of mesenchyme. It is most likely that primary intimal hyperplasia is a result of tissue growth in response to mechanical stress with contribution from the aging process and remodelling due to arterial growth. But, recurrent episodes of surface fibrin eposition and its subsequent incorporation into the intimal fibrous lesions should not be discounted. It may well be that more than one fundamental process will have to be acknowledged in this disease (55).

PROCEDURE :

MATERIALS:

The study consisted of an examination of the right coronary artery of 101 hearts removed at post-mortem examination during a five month period; June to October, 1965. These were collected from autopsies performed at the Lions Gate Hospital, Saint Paul's Hospital, Royal Columbian Hospital, Vancouver General Hospital, and Vancouver City Morgue. Table I shows the ages of the specimens used. The specimens were taken at random in the age group 0 - 30 years without regard for cause of death. Table II gives a summary of the causes of death. The hospital charts and autopsy records were examined to determine age, sex, body height and weight, heart weight, cause of death, and symptoms or signs of any associated diseases. These are summarized with the results of the quantitative examinations (Table IV).

The proximal part of the right coronary artery was removed by the pathologist performing the autopsy then fixed and preserved in 10% formal saline. Depending on the age of the individual and the gross size of the vessel the proximal one-half to one centimeter of the artery was used. On dissection of the specimens it was noted that the right coronary ostium lay near the middle of the right coronary sinus of Valsalva. In about one-half the hearts there was a second ostium about one mm. away giving rise to the conus artery. (55, 74) This artery was not used in the study. The main right coronary artery passed between the

TABLE I: AGES OF SPECIMENS USED:

AGE	SEX	
	Male	Female
Fetal	2	1
0 - 1 year	17	8
1 - 10 years	11	6
11 - 20 years	12	4
21 - 30 years	29	11
TOTAL	71	30

Table II: CAUSES OF DEATH IN THE 101 PATIENTS:

ACUTE CAUSES	
Trauma	22
Acute Infection	11
Suicide	9
Drowning	7
Prematurity	5
Ruptured berry aneurysm	4
Other causes	12
Total	70

CHRONIC CAUSES	
Malignancy	15
Congenital heart disease	4
Chronic renal disease	3
Chronic infection	3
Other causes	6
Total	31

TABLE III: CAUSES OF DEATH IN THE 0 - 1 YEAR AGE GROUP.

	NO.
Pneumonitis	6
Pneumonitis with myocarditis	1
Pneumonitis with G.I. aspiration	2
Pneumonitis with dehydration	1
Atelectasis	3
Atelectasis (stillborn)	4
Atelectasis with cerebral hemorrhage	1
Congenital heart disease	3
Hyaline membrane disease	2
Asphyxia	2
Pulmonary edema	1
Dehydration (post-op. Hirschprung's disease)	1

body of the right atrium and the main pulmonary artery into the right atrioventricular sulcus. This epicardial portion of the artery was the part used. In 10% of the human hearts the right coronary artery bifurcates near its origin and sends a large branch coursing diagnonally across the anterior free wall of the right ventricle (75). This artery was not used in the study.

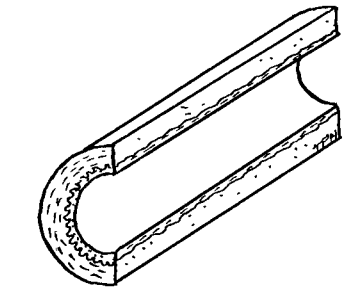
METHODS:

The removal of the proximal portion of the right coronary artery was accomplished by dissection under a dissecting microscope. The vessel was freed from the epicardial fat with gentle traction on the distal end. Since longitudinal sections were required, rather than serial sections, and as both sides were desired, the arteries were prepared in two ways depending on whether or not they had been opened at autopsy. For closed or intact vessels a ½ - 1 cm. cylinder was removed and bisected longitudinally (Figure 1 A). For vessels that had been opened, two ½ - 1 cm. ribbons from either side were prepared (Figure 1 B). In both cases it was attempted, by using the dissecting microscope, to include the areas of maximum gross intimal thickening in the part to be sectioned.

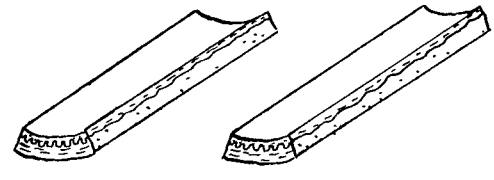
The specimens were mounted in paraffin blocks after which sections 8 microns in thickness were made and stained both with hematoxylin and eosin, and Gomori's aldehyde-fuchsin for elastic tissue.

Quantitative evaluation:

The microscopic measurements were made using an ocular micrometer.



A.



B

Figure 1: Diagramatic Representation of the Two Methods of Preparing the Tissue for Longitudinal Sectioning

All measurements were made by the author.

1. Using 10 X 10 magnification the over all length of the specimens was determined.

2. Using 10 X 10 or 10 X 40 magnification, depending on the size of the arterial wall, the thickness of the intima from the internal elastic lamina to the endothelium was measured (Figure 2). Measurements were taken at approximately one millimeter intervals.

3. At the same intervals the thickness of the media, from internal elastic lamina to adventitia was measured (Figure 2).

4. Using 10 X 40 magnification, the amount of elastic change was measured for the whole length. A solid regular, wavy band was interpreted as normal. Areas of disruption, fragmentation, fraying, splitting and swelling were considered "change". (Figure 2).

Steps 1 to 4 were repeated for the second side of the artery.

Of the 101 specimens, seven showed some areas of advanced atheroma. These areas have been excluded in calculating the intimal thickness as the underlying media has been partially, or in some cases almost completely, destroyed, which would give a distorted picture of the intimal thickness. Since there was very little atheroma in the specimens, there was very little non-uniform distortion due to shrinkage during fixation.

The data was analyzed by the Digital Computer to derive the equations of the best straight line approximation and the correlation co-efficients.

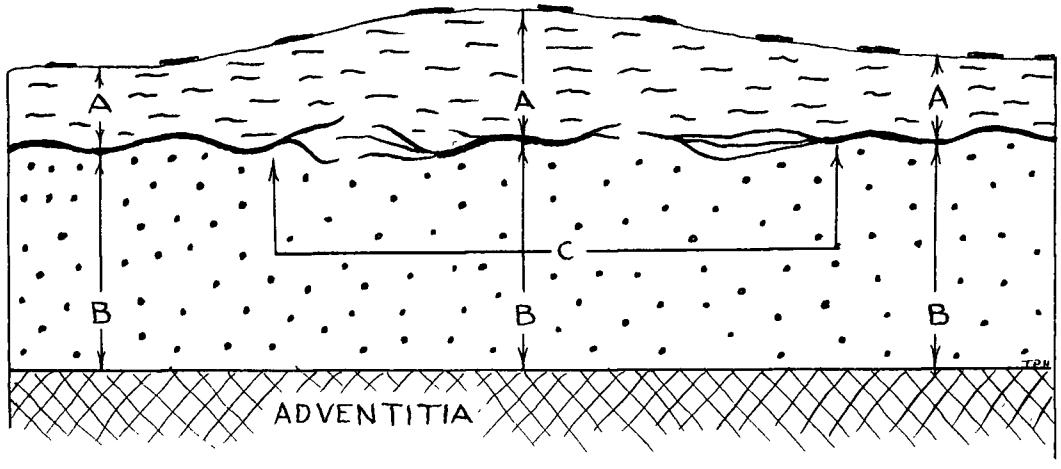


Figure 2 : Diagram of longitudinal section to show quantitative evaluation:

A. 1 mm. interval measurements of intimal thickness

B. 1 mm. interval measurements of medial thickness

C. area of internal elastic lamina change

RESULTS :

The measurements of the intimal thickness were averaged and are presented in Table IV as "average thickness of intima" expressed in microns. Similarly the measurements for medial thickness are presented as "average thickness of media ". The intima has also been expressed as a percentage of the total arterial wall (intimal thickness + medial thickness) and is presented in column 5 in Table IV. The internal elastic lamina change is expressed as a percentage of the total length used of the artery (column 6, Table IV). For statistical analysis the causes of death have been divided into chronic and non-chronic diseases. These are explained in Table II.

Since only 21 of the individual specimens had recorded blood pressures, this parameter cannot be used in analyzing the data.

Figures 3 to 9 are photomicrographs to demonstrate the different aspects of the arterial wall in the different age groups studied.

Figure 3 is a hematoxylin and eosin stained preparation of a longitudinal section of the right coronary arterial wall as seen in most neonatal infants. This particular specimen is from a 0.25 year old white female infant who died of dehydration due to acute enteritis (specimen number A65-432). There is a normal or intact internal elastic lamina underlying a thin endothelial lining (not demonstrated well in the photograph). Under the internal elastic lamina are regular circularly arranged smooth

TABLE IV: DATA OBTAINED FROM THE 101 SPECIMENS

AGE	SEX	AVERAGE THICKNESS OF INTIMA (Microns	AVERAGE THICKNESS OF MEDIA(microns)	% INTIMA OF TOTAL	% INTERNAL ELASTIC LAMINA CHANGE	WEIGHT (lbs)	HEART WEIGHT (grams)	CAUSE OF DEATH
6.50	M	50.57	142.04	26.11	21.80	41.50	95	CHRONIC
25.00	M	145.08	161.48	47.39	48.03	180.00	370	NON-CHRONIC
9.00	F	53.02	98.90	35.42	23.66		110	NON-CHRONIC
1.58	F	37.23	113.85	24.67	19.76	21.50	65	CHRONIC
30.00	M	41.42	125.51	24.81	85.75		550	CHRONIC
0.01	M	6.51	39.10	14.27	26.75	3.50	16	NON-CHRONIC
0.25	F	6.80	53.04	12.63	17.16	12.00	50	NON-CHRONIC
0.01	M	23.09	108.38	17.02	70.38	12.08	64	CHRONIC
1.25	M	44.88	102.00	30.55	29.82	22.00	40	CHRONIC
3.50	M	89.25	105.28	45.43	69.78	24.00	192	CHRONIC
11.00	M	37.18	110.64	24.86	21.01		150	CHRONIC
0.12	M	59.79	107.64	35.38	81.82	6.62	25	CHRONIC
16.00	F	67.99	166.60	28.98	22.76	110.00	230	NON-CHRONIC
6.00	M	28.41	96.85	20.87	11.89	55.25	140	CHRONIC
0.06	F	0.00	55.25	0.00	0.27	7.34	34	CHRONIC
29.00	M	136.20	113.04	51.84	56.56		250	CHRONIC
0.00	M	0.00	10.25	0.00	0.00	2.33	7	NON-CHRONIC
27.00	M	520.50	227.40	69.63	85.15	104.00	300	CHRONIC
0.13	M	72.84	50.74	46.63	83.51	5.14	31	NON-CHRONIC
25.00	M	170.40	236.30	41.90	80.74	145.00	590	CHRONIC
7.00	M	37.73	83.35	31.20	49.64	50.00	140	CHRONIC
24.00	F	80.14	97.14	44.97	32.74		250	NON-CHRONIC
25.00	M	153.46	131.65	53.81	64.00		240	CHRONIC
5.50	M	55.97	122.54	30.05	57.65	47.00	190	CHRONIC
0.03	M	42.21	49.37	33.37	45.94	6.25	24	NON-CHRONIC
1.08	M	21.30	121.55	15.95	44.80	19.16		CHRONIC
0.00	F	2.67	34.73	7.08	7.90	2.95	7	NON-CHRONIC
8.00	M	74.80	189.04	27.71	59.60	80.00	190	CHRONIC
17.00	M	75.94	222.22	25.25	42.18	120.00	225.	CHRONIC
21.00	F	49.30	188.12	20.76	51.99	160.00	300	NON-CHRONIC
0.08	F	50.51	31.08	61.90	100.00	4.21	8	NON-CHRONIC
4.00	M	164.40	156.70	51.90	97.50	35.00	180	CHRONIC
16.00	M	112.47	241.03	31.79	98.00	160.00	500	CHRONIC
24.00	F	25.50	43.60	36.90	15.80	90.00	220	NON-CHRONIC

TABLE IV: DATA OBTAINED FROM THE 101 SPECIMENS (CONT'D.)

AGE	SEX	AVERAGE THICKNESS OF INTIMA (Microns)	AVERAGE THICKNESS OF MEDIA (microns)	% INTIMA OF TOTAL	INTERNAL ELASTIC LAMINA CHANGE %	WEIGHT (lbs)	HEART WEIGHT (grams)	CAUSE OF DEATH
3.50	M	26.07	91.80	22.11	30.87%	23.00	60	CHRONIC
15.00	F	107.25	130.55	45.17	29.01		210	CHRONIC
11.00	M	103.20	165.83	35.17	78.64	90.00		NON-CHRONIC
0.25	F	22.10	87.83	20.10	20.17			NON-CHRONIC
34.00	M	224.00	187.10	54.03	35.87	185.00	450	NON-CHRONIC
1.00	F	28.05	79.04	26.02	32.43	20.00		NON-CHRONIC
27.00	M	37.12	202.02	15.66	31.86	145.00	420	NON-CHRONIC
7.00	F	11.76	83.02	12.40	7.06	65.00		NON-CHRONIC
8.00	F	18.50	100.43	15.34	12.16	70.00	123	NON-CHRONIC
21.00	M	141.67	221.56	39.00	61.85		360	NON-CHRONIC
22.00	F	49.30	136.85	26.32	34.31	125.00	270	NON-CHRONIC
33.00	F	94.17	134.98	41.01	49.65		260	NON-CHRONIC
22.00	F	34.00	133.73	19.74	37.60	135.00		NON-CHRONIC
0.67	F	23.63	75.26	24.22	24.83	20.19		NON-CHRONIC
0.12	M	52.02	139.40	25.19	94.70	7.87	37	CHRONIC
29.00	M	73.38	144.43	32.20	52.65	175.00	340	NON-CHRONIC
25.00	M	130.61	170.57	43.45	95.19	135.00	250	NON-CHRONIC
23.00	F	257.93	119.32	68.49	96.98	150.00	220	NON-CHRONIC
28.00	M	158.39	218.09	41.29	58.23	175.00	250	NON-CHRONIC
28.00	M	189.20	185.90	50.41	75.69	225.00	310	NON-CHRONIC
18.00	M	118.07	141.06	44.33	82.70	140.00	220	NON-CHRONIC
28.00	M	119.63	221.10	35.12	85.33	145.00	420	NON-CHRONIC
21.00	F	75.28	129.68	36.72	67.93	125.00	240	NON-CHRONIC
25.00	M	67.08	125.82	34.71	43.91	170.00	250	NON-CHRONIC
30.00	M	431.40	293.48	57.50	100.00	155.00	480	CHRONIC
20.00	M	139.70	200.20	41.01	91.50	190.00	325	NON-CHRONIC
0.20	M	9.07	83.57	9.90	24.16	10.19		NON-CHRONIC
24.00	F	91.56	192.09	32.17	100.00	135.00	300	NON-CHRONIC
16.00	F	129.63	207.83	38.50	100.00	120.00	240	NON-CHRONIC
22.00	M	218.20	184.99	51.38	96.35	190.00	210	NON-CHRONIC
27.00	M	81.41	135.56	36.89	83.60	130.00	300	NON-CHRONIC
23.00	M	431.60	214.91	64.90	98.50	170.00	230	NON-CHRONIC
15.00	M	138.26	164.33	45.69	89.51	130.00	300	CHRONIC
30.00	M	257.13	157.51	61.62	92.47	180.00	450	NON-CHRONIC.

TABLE IV: DATA OBTAINED FROM THE 101 SPECIMENS (CONT'D.)

AGE	SEX	AVERAGE THICKNESS OF INTIMA(microns)	AVERAGE THICKNESS OF MEDIA(microns)	% INTIMA OF TOTAL	% INTERNAL ELASTIC LAMINA CHANGE	WEIGHT (lbs)	HEART WEIGHT (grams)	CAUSE OF DEATH
26.00	M	110.50	175.95	38.33	98.09	200.00	280	NON-CHRONIC
24.00	M	145.02	308.11	32.00	100.00	165.00	300	NON-CHRONIC
33.00	M	173.25	171.32	50.28	100.00	200.00	300	NON-CHRONIC
0.71	M	0.00	82.13	0.00	0.00	15.00	40	CHRONIC
0.00	M	13.36	32.78	28.49	45.19	3.00	10	NON-CHRONIC
3.50	F	24.58	102.91	18.89	66.91		100	CHRONIC
0.00	F	2.67	56.34	4.38	22.09	9.11	35	NON-CHRONIC
22.00	F	84.72	86.42	50.51	56.70	158.00	300	NON-CHRONIC
0.02	M	0.74	32.55	2.22	26.71	15.93	24	NON-CHRONIC
0.00	F	0.00	36.27	0.00	0.00	6.05	50	NON-CHRONIC
18.00	M	177.95	212.71	45.31	97.50		320	NON-CHRONIC
0.00	M	0.00	38.08	0.00	8.15	6.62	18	NON-CHRONIC
0.00	F	5.51	55.25	9.17	22.83		20	NON-CHRONIC
0.17	M	0.00	70.30	0.00	10. 25	13.93	40	CHRONIC
0.00	M	2.04	52.02	3.80	35.29		20	NON-CHRONIC
21.00	M	69.20	190.83	32.59	97.50			NON-CHRONIC
20.00	M	183.33	157.91	53.67	96.55		275	CHRONIC
0.00	M	16.31	41.29	27.61	85.50	7.90		NON-CHRONIC
23.00	M	66.00	154.00	29.26	73.55	170.00		NON-CHRONIC
0.21	M	1.15	57.95	1.91	12.47			NON-CHRONIC
21.00	M	204.05	180.04	53.12	100.00			NON-CHRONIC
18.00	M	62.32	163.93	27.56	73.30			NON-CHRONIC
16.00	M	99.17	149.03	39.96	78.80		375	NON-CHRONIC
21.00	F	71.68	137.76	33.95	69.37			NON-CHRONIC
0.17	M	2.92	69.90	4.02	17.40			NON-CHRONIC
27.00	M	250.21	135.99	64.84	100.00	200.00		NON-CHRONIC
17.00	M	104.69	199.31	34.49	97.90	175.00	450	NON-CHRONIC
15.00	F	61.55	127.15	32.62	86.48	115.00	270	NON-CHRONIC
0.06	M	4.53	81.03	4.94	58.05	9.12	10	NON-CHRONIC
29.00	M	88.83	118.58	42.70	74.64	160.00	420	NON-CHRONIC
3.00	M	56.21	163.32	25.58	96.75	32.00	68	CHRONIC
23.00	M	132.83	188.65	41.30	80.09	145.00	250	NON-CHRONIC
0.13	M	146.94	109.08	55.96	78.72			NON-CHRONIC

muscle cells of the media. This is an example of the "normal" wall of a muscular artery.

Figure 4 is the same specimen as figure 3 stained for elastic tissue with aldehyde fuchsin. This photograph demonstrates better the normal internal elastic lamina. Scattered elastic fibers are seen also in the media and adventitia. The average intimal thickness in this specimen is 7 microns, and the average medial thickness is 53 microns.

Figure 5 is a longitudinal section, stained with aldehyde fuchsin, of the right coronary arterial wall in a two year old white male who died from acute blast cell leukemia (specimen number A65-431). There is a minimal proliferation of connective tissue elements between the endothelium and the internal elastic lamina, which shows only minimal change. The internal elastic lamina shows evidence of splitting in the upper right corner with two definite breaks in the central area, under the area of maximal intimal hyperplasia. The intima shows evidence of elastic neogenesis and a longitudinal arrangement of the fibers. In this specimen the average intimal thickness is 37 microns, the average medial thickness is 114 microns, and the average change of the internal elastic lamina is 19% of the total length.

Figure 6 is a longitudinal section, stained with hematoxylin and eosin, of the right coronary arterial wall in a 25 year old white male who died of a ruptured berry aneurysm (specimen number A65-409). There is a moderate amount of intimal hyperplasia. The average thickness of the intima is 145 microns and the average thickness of the media is 161 microns.

Figure 3. An intact internal elastic lamina overlying the media composed of regularly circularly arranged smooth muscle cells. Longitudinal section, Paraffin, H & E., 50 X.

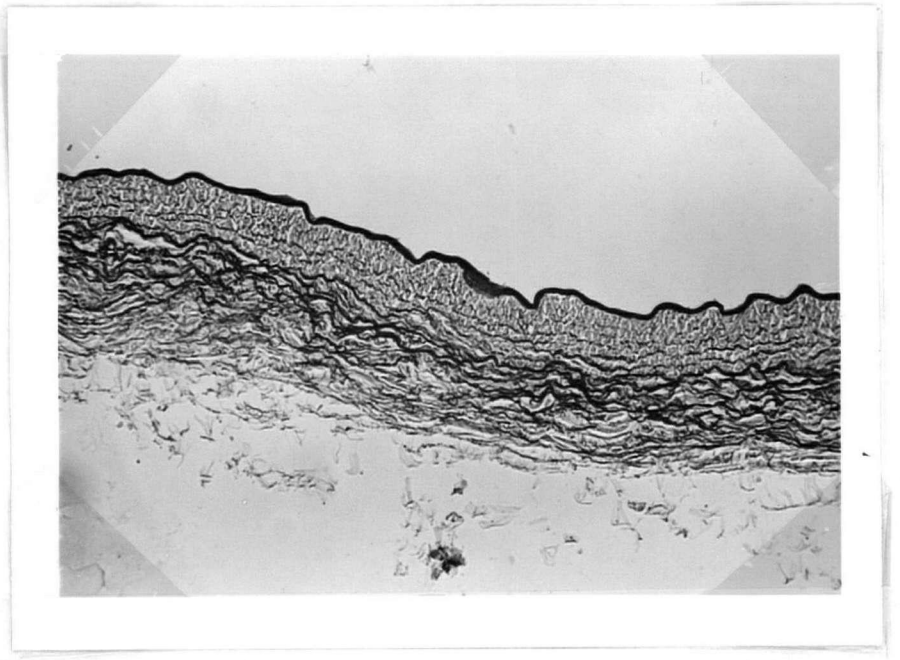


Figure 4. Same specimen as figure 3. Longitudinal section, Paraffin, Aldehyde Fuchsin, 50 X.

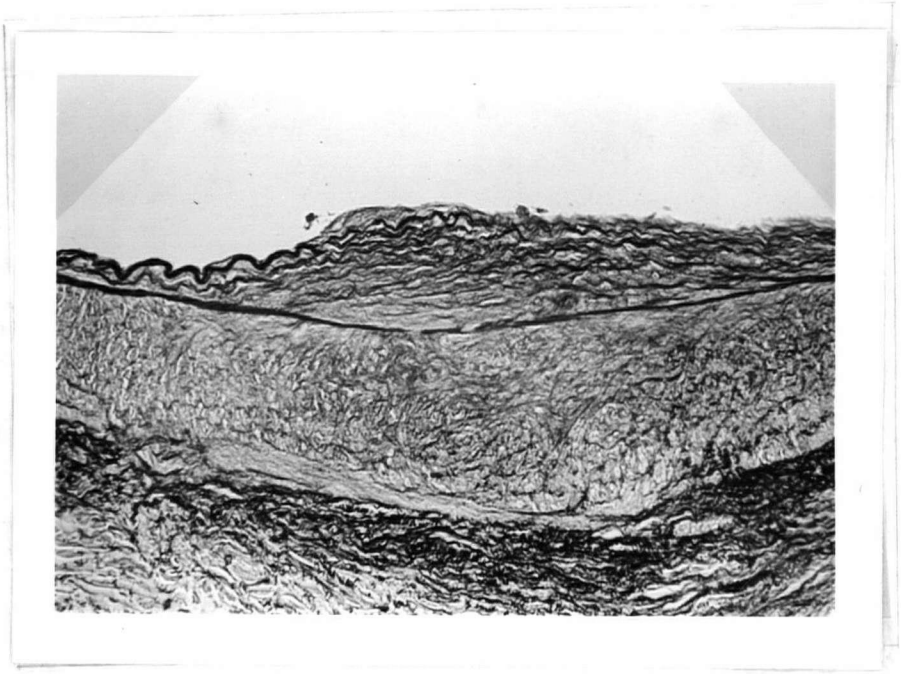


Figure 5. Early intimal hyperplasia, most marked over the area of most internal elastic lamina change. Note the gaps and area of fraying of the internal elastic lamina, and the elastic neogenesis in the intima. Longitudinal section, Paraffin, Aldehyde, Fuchsin, 50 X.

This photograph demonstrates well, the reorientation of the smooth muscle cells which are longitudinally arranged in the intima. The internal elastic lamina demonstrates a moderate degree of change.

Figure 7 is the same specimen as Figure 6 stained with aldehyde fuchsin. The site is not exactly the same, as the large break in the internal elastic lamina seen in the centre of the hematoxylin and eosin preparation is on the right in this photograph. This photograph demonstrates more clearly a moderate degree of change in the internal elastic lamina. There are two large gaps present. The average change from the normal of the internal elastic lamina in this specimen is 48% of the total length.

Figures 8 and 9 are both examples of a large amount of intimal hyperplasia and a large degree of internal elastic lamina change. Both are longitudinal sections of the right coronary artery stained with aldehyde fuchsin.

Figure 8 is a four year old white male who died following open heart surgery for repair of an interventricular septal defect (specimen number A65-434). The average intimal thickness is 164 microns. The average medial thickness is 156 microns. There are only fragments of normal internal elastic lamina remaining in the specimen.

Figure 9 is a 20 year old white male who died of cerebral trauma (specimen number 65-809). The average intimal thickness is 154 microns. The average medial thickness is 200 microns.

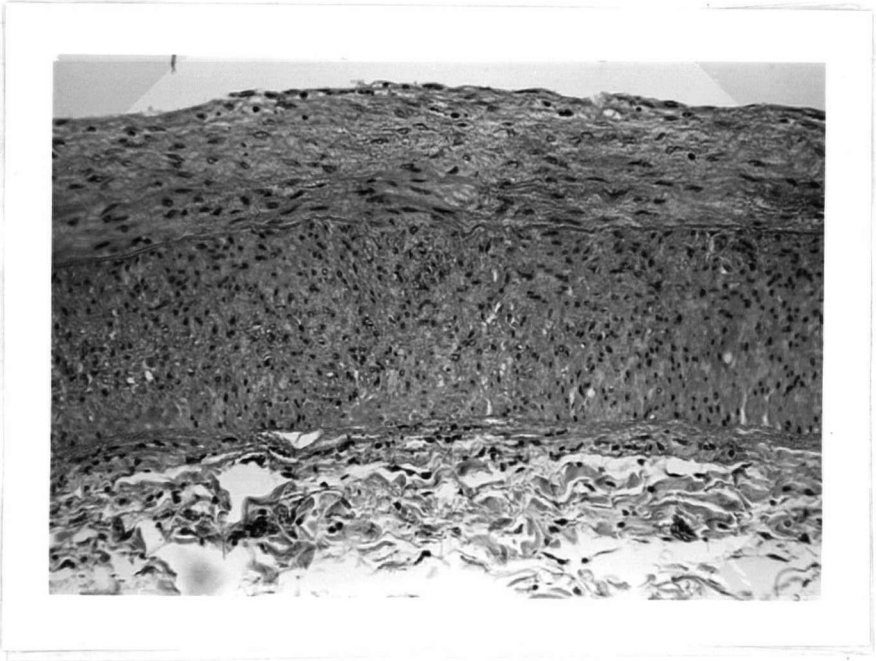


Figure 6. A moderate amount of intimal thickening is seen over an internal elastic lamina which demonstrates large gaps. Note the longitudinally arranged smooth muscle cells, especially in the basal part of the intimal layer. Longitudinal section, Paraffin, H. & E.,50 X.

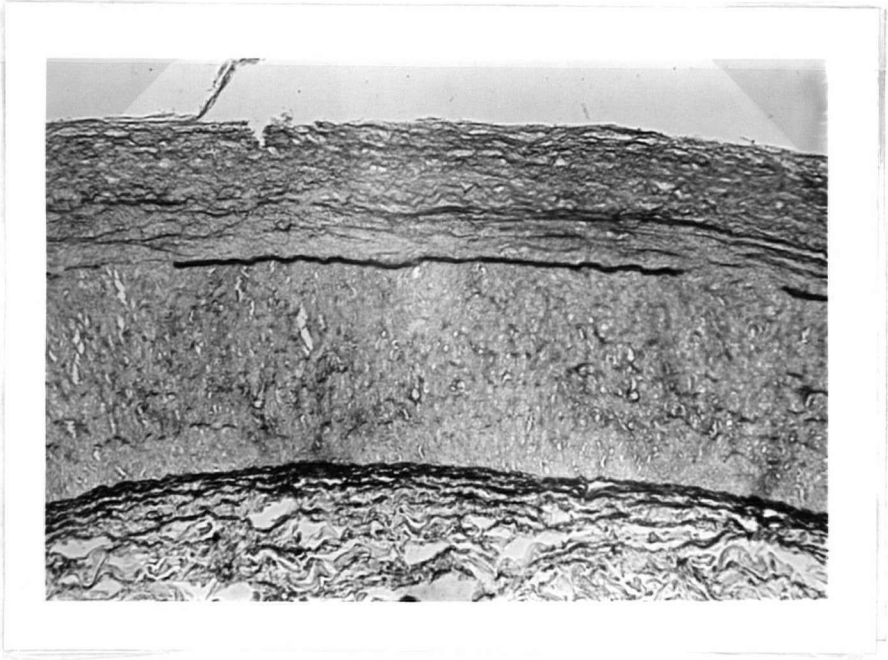


Figure 7. Same specimen as figure 6. Longitudinal section, Paraffin, Aldehyde Fuchsin, 50 X.

There is no normal internal elastic lamina remaining in the specimen.

Although this series of seven examples does not conform to the relationships found in regard to intimal thickness vs. age, and internal elastic lamina change vs. age, as shown in Figures 11, and 16, they do illustrate the relationship of intimal hyperplasia vs. internal elastic lamina change as shown in Figures 13, and 14, and 15.

Figure 5 demonstrates an interesting observation which was noted in specimens where the intimal thickness was minimal and somewhat irregular. In these specimens the maximum intimal thickness almost always occurred over the areas of greatest internal elastic lamina change. Because of the relatively few specimens in this category, no statistical correlation was attempted. Also, the ratio of cellular to extracellular material was noted to be higher in the early lesions when compared to later ones.

The qualitative and quantitative results of Table IV are presented in graphic and tabular form in the suceeding pages.

Figure 10 is a graph of the 101 observations obtained for the absolute thickness of the intima in microns, on the Y axis, compared to the age in years, on the X axis. The equation of the best straight line approximation is:
$Y = -89.88 + 13.25X$. This line does not pass through the origin of a distortion resulting from three very large values for the intimal thickness. These are the three values at the top of the graph which range between 431 and 520 microns. However, from the spread of points on the graph it is evident that

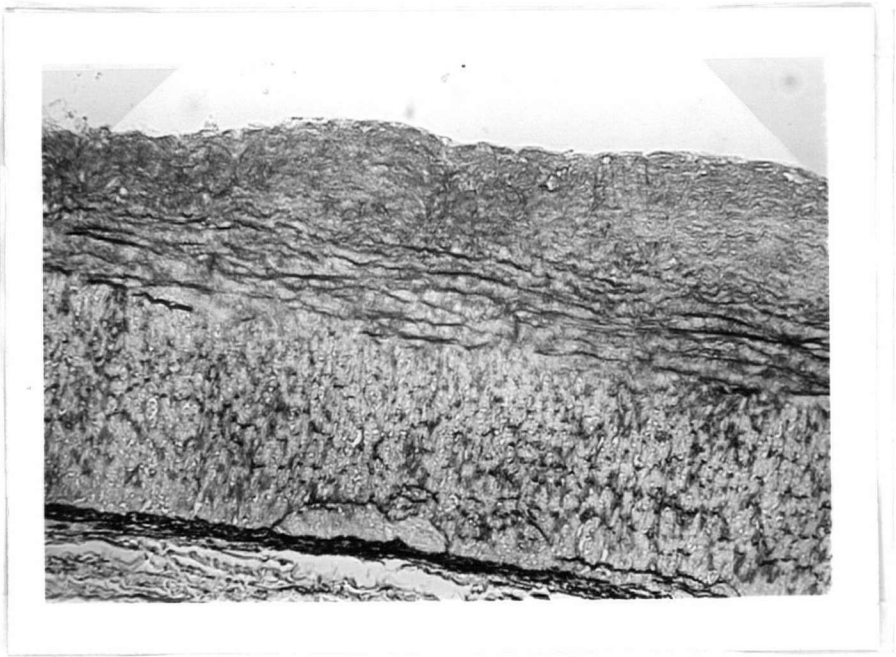


Figure 8. There is a thick intimal layer with only remnants of the internal elastic lamina remaining between it and the medial layer. Note the elastic neogenesis in the base of the intima. Longitudinal section, Paraffin, Aldehyde Fuchsin, 50 X.

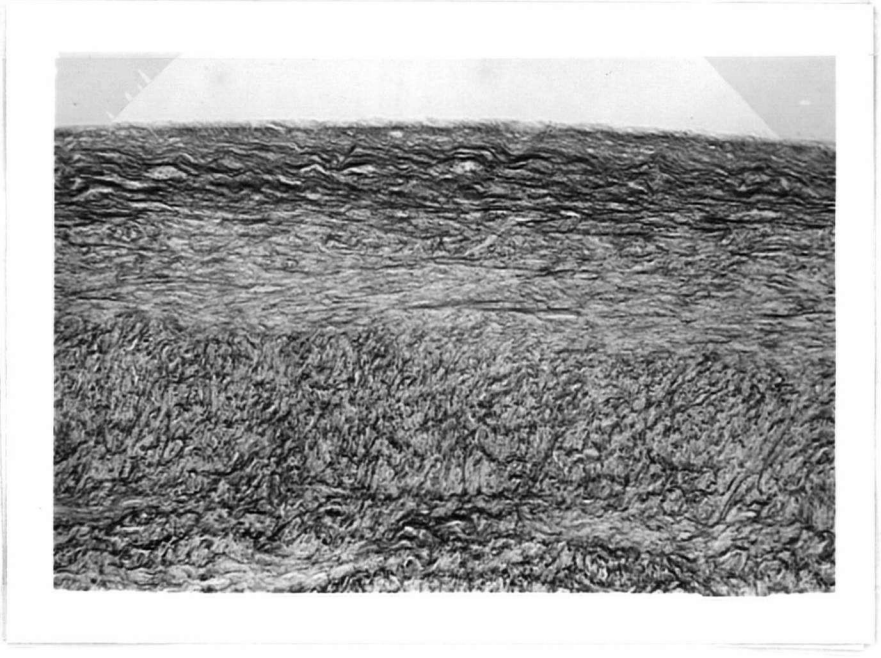


Figure 9. There is a thick intimal layer with no internal elastic lamina remaining. The boundary between the intimal and medial layers is taken as the zone of reorientation of the fibers which is clearly seen here. Longitudinal section, Paraffin, Aldehyde Fuchsin, 50 X.

there is correlation between these two variables. The correlation co-efficient r is 0.61 ($p < .01$, error df 99). There is, therefore a highly significant correlation between these two variables.

Figure 11 is a graph of the absolute thickness of the intima, on the Y axis, compared to the age in years, on the X axis, for patients dying of non-chronic causes (70 observations). It is evident from the graph that there is a similar relationship as for Figure 10. Although only a linear regression was calculated for Figure 10, both these graphs suggest that higher powers should be employed to obtain a more accurate approximation to the data, which would therefore give an even better correlation co-efficient. An approximation of this is superimposed on Figure 11.

Figure 12 is a graph of the relative thickness of the intima, on the Y axis, (intima expressed as a percentage of the total thickness of the arterial wall) compared to the age in years, on the X axis (101 observations). This does not correlate as well as figure 10 due to the artificially high values in the infants where any intimal thickness is magnified on a relative scale due to the thinness of the media in this age group.

In Table V, the specimens are divided into three arbitrary age groups; 0-2, 1-15, & 16-30 years. The average intimal thickness in each group is compared using a paired sample "T" test. From this table it is seen that there is a highly significant difference between the three age groups. This is additional proof that the intimal thickness does increase with age.

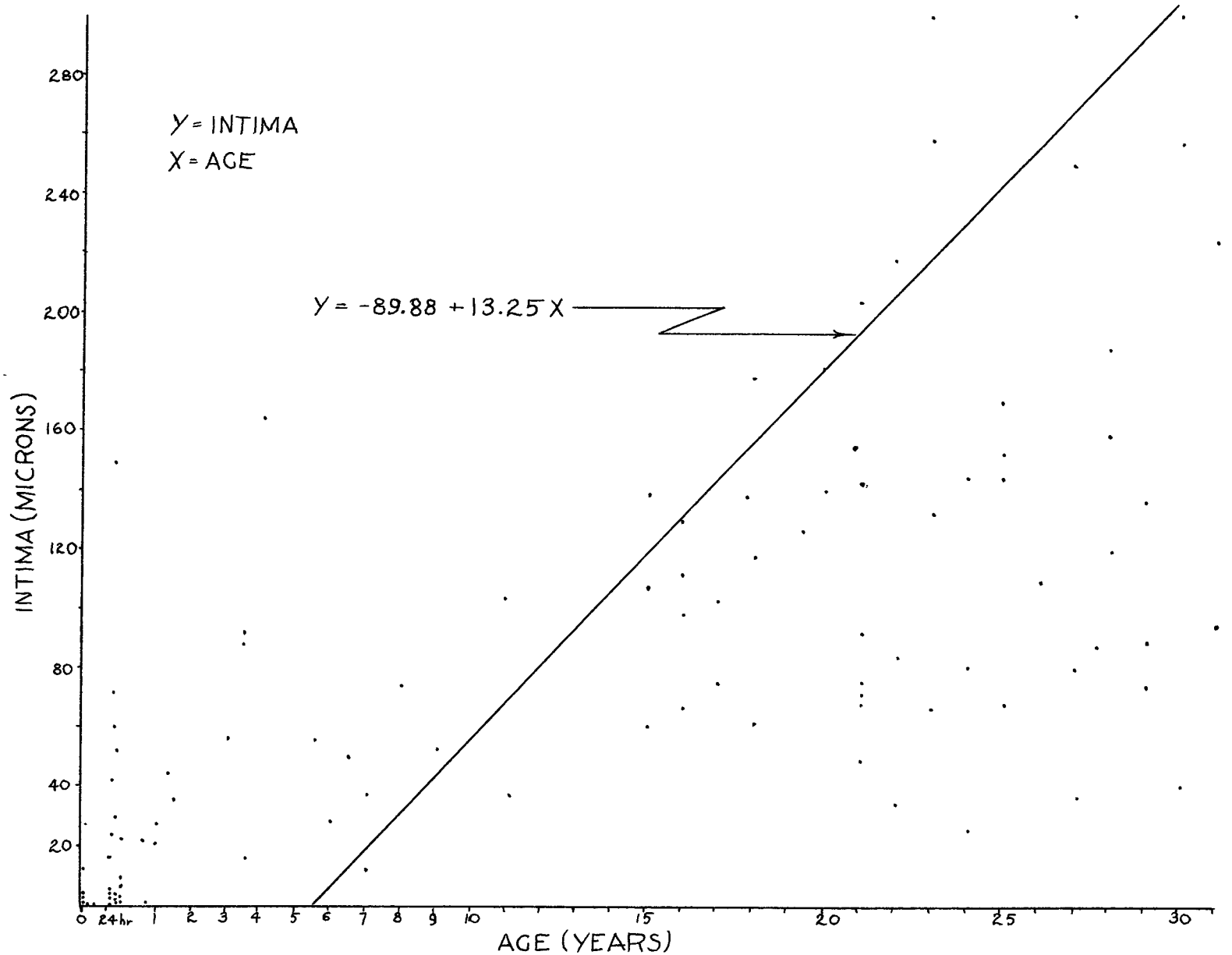

Y = INTIMA
X = AGE
Y = -89.88 + 13.25 X
INTIMA (MICRONS)
280
240
200
160
120
80
40
20
0
24hr
1
2
3
4
5
6
7
8
9
10
15
20
25
30
AGE (YEARS)

FIGURE 10

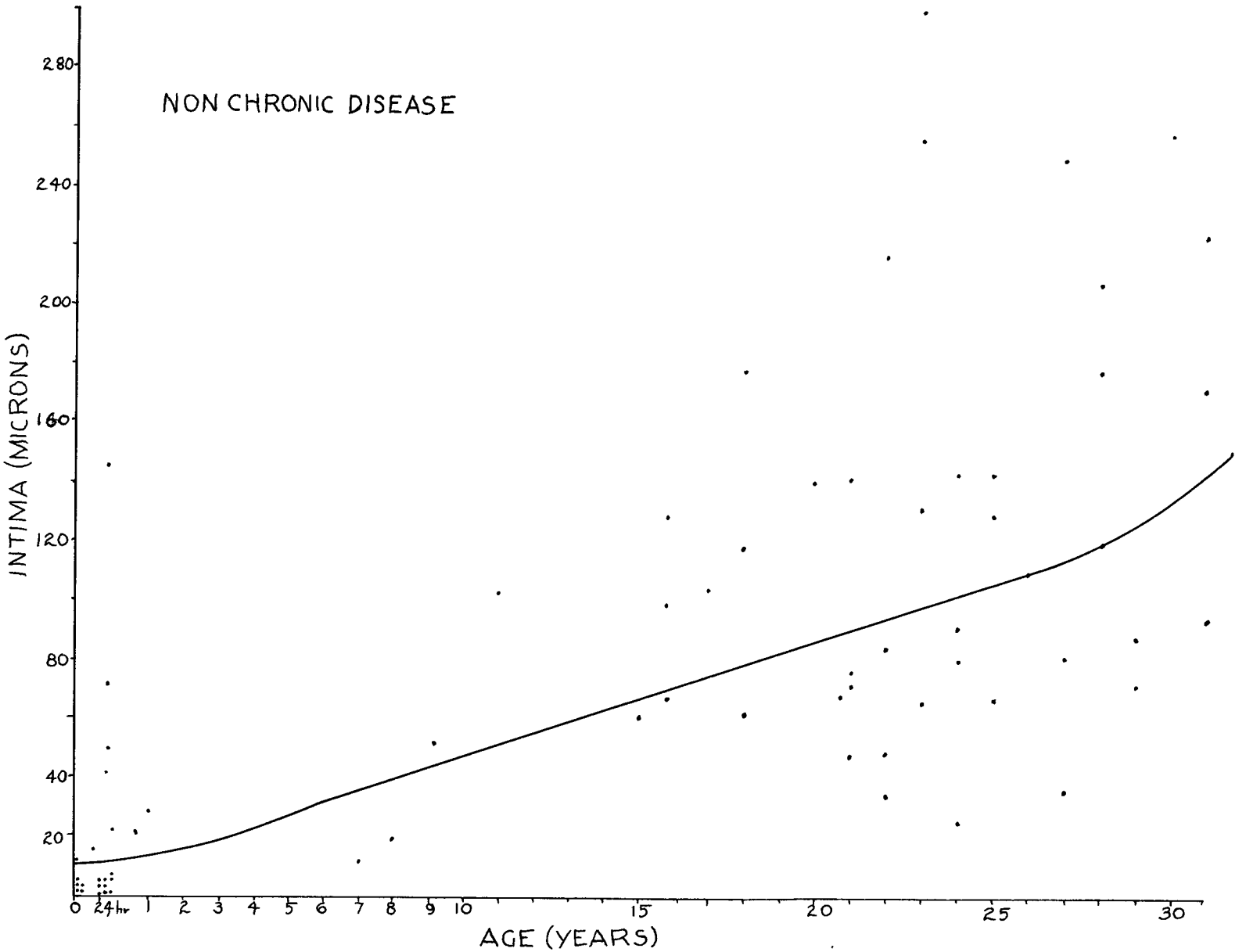

NON CHRONIC DISEASE
INTIMA (MICRONS)
280
240
200
160
120
80
40
20
0
24 hr
1
2
3
4
5
6
7
8
9
10
15
20
25
30
AGE (YEARS)

FIGURE 11

FIGURE 12

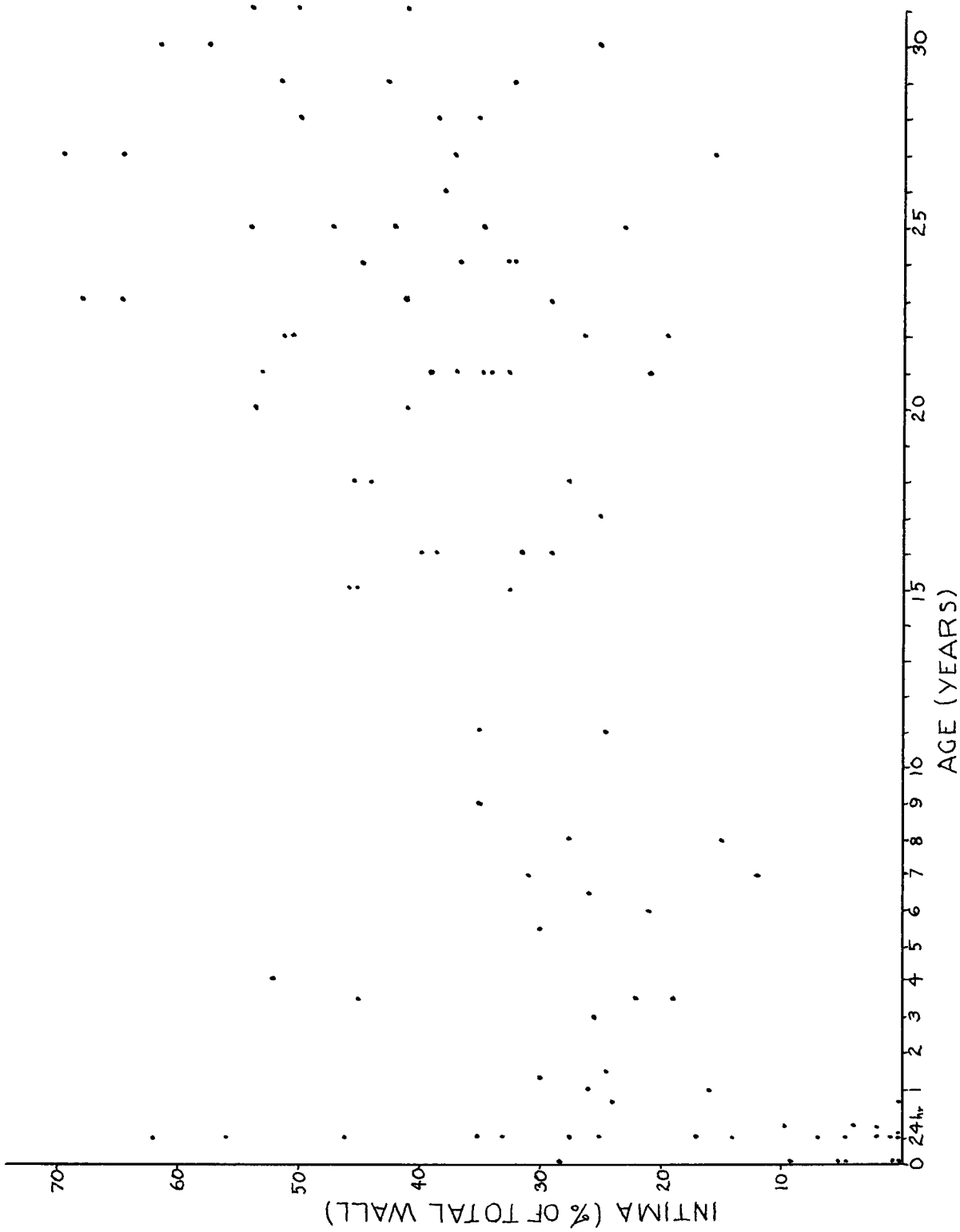

INTIMA (% OF TOTAL WALL)
70
60
50
40
30
20
10
0
24hr
1
2
3
4
5
6
7
8
9
10
15
20
25
30
AGE (YEARS)

TABLE V. Comparison of intimal hyperplasia in three age groups. (sexes combined)

AGE	AVERAGE INTIMAL THICKNESS (u)	CALCULATED STUDENT'S T	SIGNIFICANCE $\alpha = .05$
0-2 1-15	17.09 57.72	t= 4.811 df 51	significant difference $p < .001$
1-15 16-30	57.72 140.24	t= 3.713 df 71	significant difference $p < .001$

TABLE VI. Comparison of intimal hyperplasia in three age groups. (Males)

AGE	SAMPLE SIZE	AVERAGE INTIMAL THICKNESS (u)	CALCULATED STUDENT'S T	SIGNIFICANCE $\alpha = .05$
0-2 1-15	20 14	18.64 66.28	t=6.456 df32	significant difference $p < .001$
1-15 16-30	14 38	66.28 158.29	t=3.353 df50	significant difference $p < .01$

TABLE VII. Comparison of intimal hyperplasia in three age groups. (Females

AGE	SAMPLE SIZE	AVERAGE INTIMAL THICKNESS (u)	CALCULATED STUDENT'S T	SIGNIFICANCE $\alpha = .05$
0-2 1-15	10 8	15.71 42.74	t=2.320 df 16	significant difference $.01 < p < .05$
1-15 16-30	8 13	42.74 85.41	t=1.890 df 19	no significant difference $.05 < p < .10$

In tables VI and VII the three age groups are divided into males females. Table VI demonstrates that in males there is still a highly significant difference between the three groups. However, in table VII it is seen that while there is in females a significant difference at the 95% level between the 0-2 and the 1-15 year age groups, there is not a significant difference between the 1-15 and 16-30 year age groups. This may be a reflection of the smaller sample sizes in the female groups.

If these same three groups are used to compare differences in intimal thickness between the sexes, it is seen in table VIII that no significant difference exists between the males and females in the 0-2 and 1-15 year age groups but that there is a significant difference in the 16-30 year age groups. Again this may be a reflection of sample size as there are very few females in the 16-30 age groups compared to the number of males.

TABLE VIII. Comparison of intimal thickness in male and female.

AGE	INTIMAL THICKNESS (AV.)		CALCULATED STUDENT'S T	SIGNIFICANCE $\alpha = .05$
	MALE	FEMALE		
0-2	18.64	15.71	t=0.350 df 28	no significant difference $p < .05$
1-15	66.28	42.74	t=1.348 df 20	no significant difference $.20 < p < .30$
16-30	158.99	85.41	t=2.373 df 49	significant difference $.02 < p < .05$

Figure 13 is a graph of the absolute thickness of the intima, on the Y axis, compared with the internal elastic lamina change, on the X axis (101 observations). The equation of the best straight line approximation is: $Y=8.07+1.40\ X$. The correlation coefficient r is 0.49 ($p < .01$, error df 99). There is therefore, significant correlation between these two variables.

Figure 14 is the same as figure 13 but uses only data from patients dying of non-chronic disease. The regression and correlation have not been calculated but it is evident from the graph that correlation is at least as good as that of figure 13. There are relatively fewer specimens with a small intimal thickness and a high degree of internal elastic lamina change, which suggests that perhaps disease processes do have some effect on the elastic changes.

Figure 15 is a graph of the relative intimal thickness, on the Y axis, compared to the amount of internal elastic lamina change, on the X axis (101 observations). This relationship is used as well as that of figure 13 because this is a comparison of two relative effects of tissue change. The equation of the best straight line approximation is : $Y=11.75+0.35\ X$. The correlation coefficient r is 0.66 ($p < .01$, error df 99). This is a highly significant correlation between these two variables. It is much better than that in figure 13.

Figure 16 is a graph of the amount of internal elastic lamina change, on the Y axis, compared to the age in years, on the X axis (101 observations). The equation of the best straight line

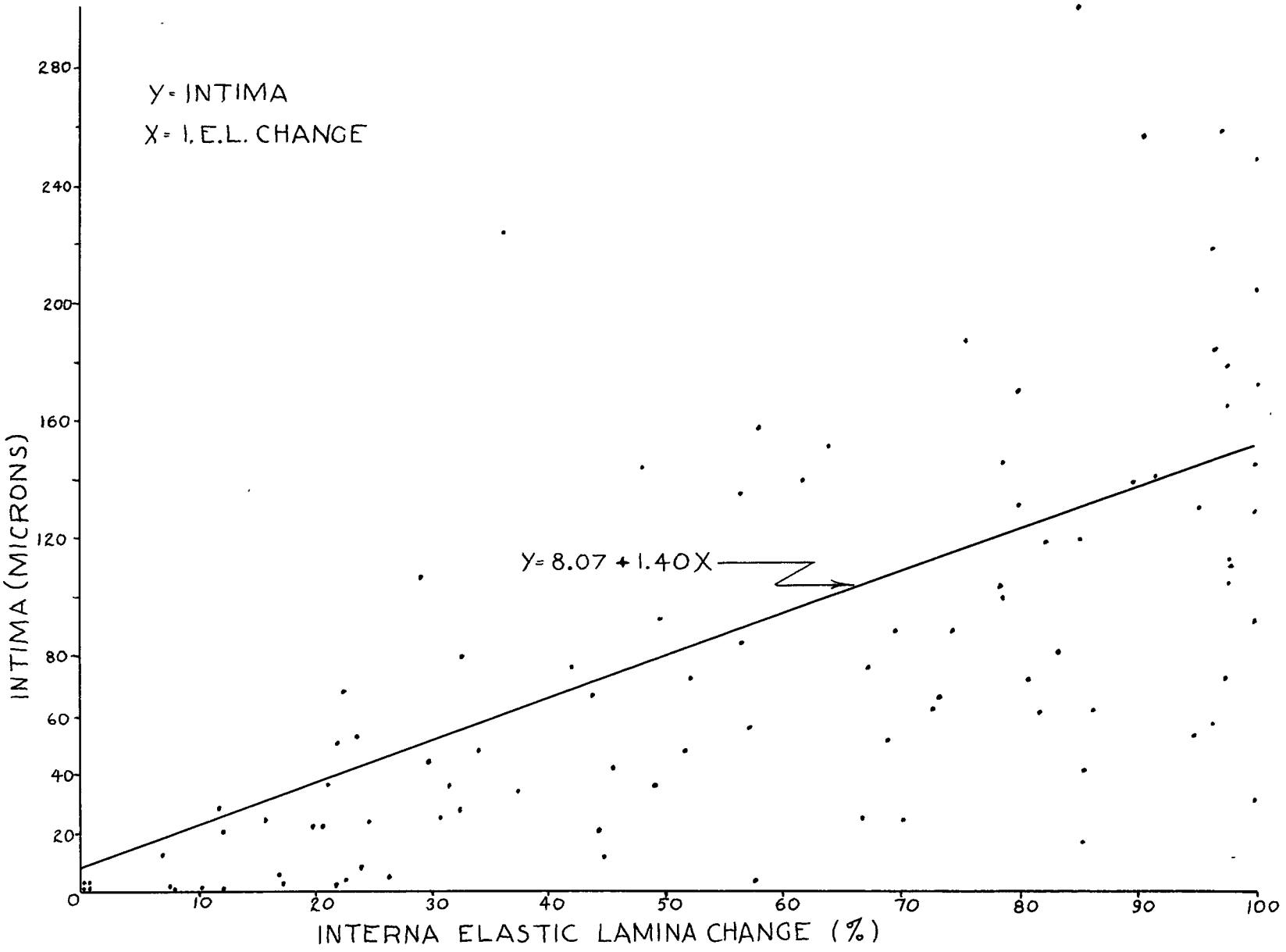

Y = INTIMA
X = I.E.L. CHANGE
Y = 8.07 + 1.40X
INTIMA (MICRONS)
INTERNA ELASTIC LAMINA CHANGE (%)
0
20
40
60
80
120
160
200
240
280
10
20
30
40
50
60
70
80
90
100

FIGURE 13

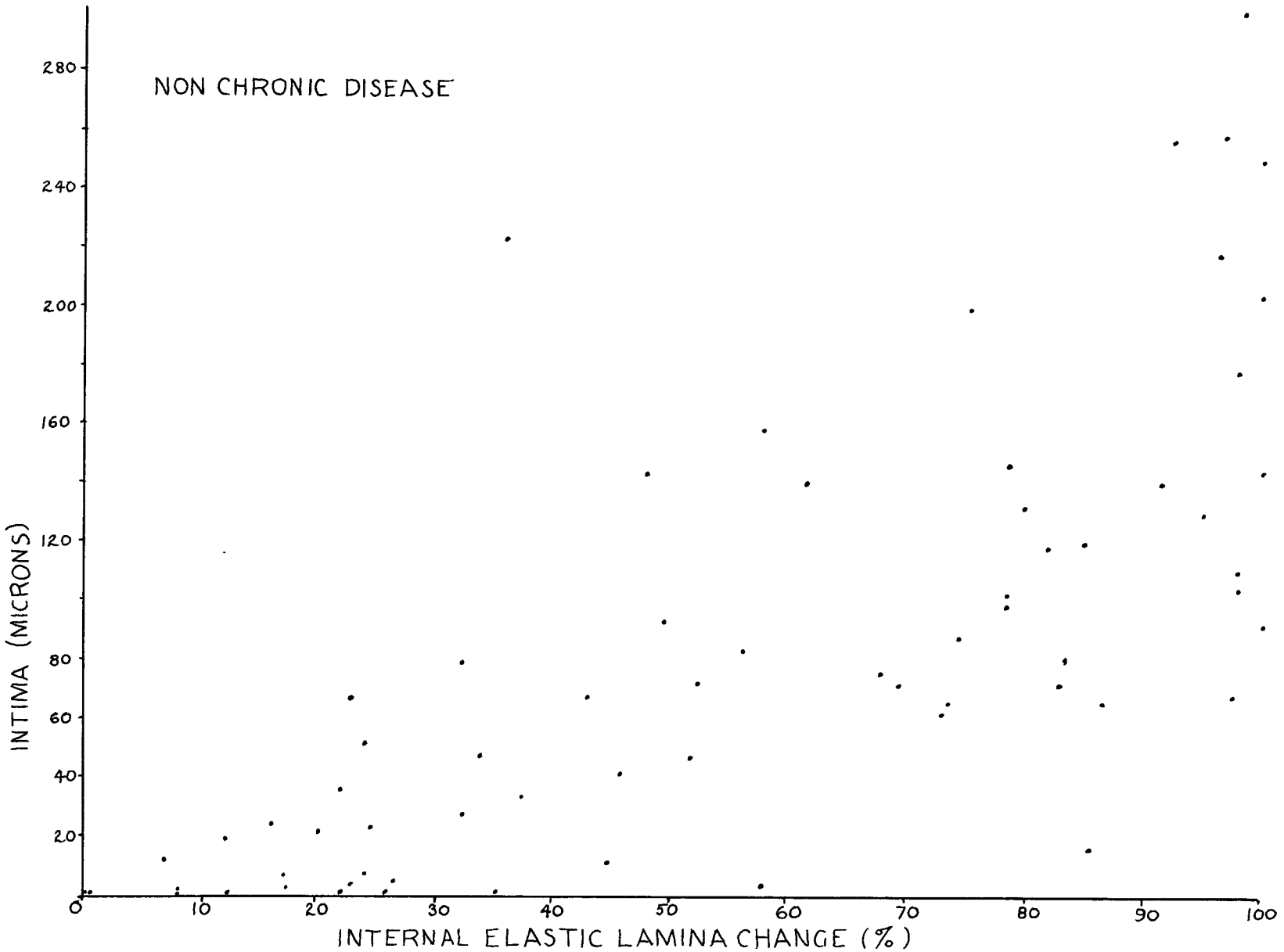

NON CHRONIC DISEASE
INTIMA (MICRONS)
280
240
200
160
120
80
60
40
20
0
10
20
30
40
50
60
70
80
90
100
INTERNAL ELASTIC LAMINA CHANGE (%)

FIGURE 14

FIGURE 15

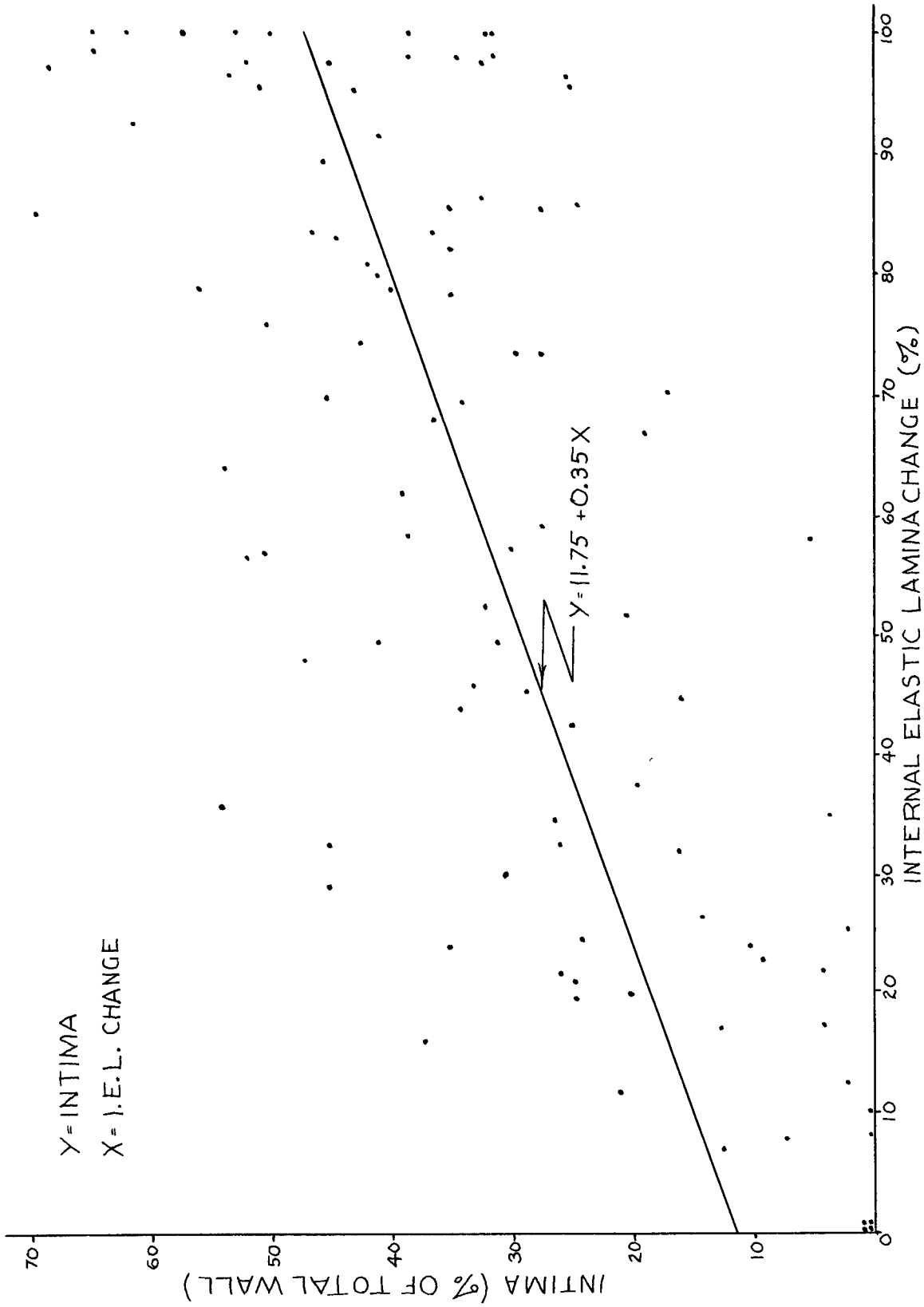
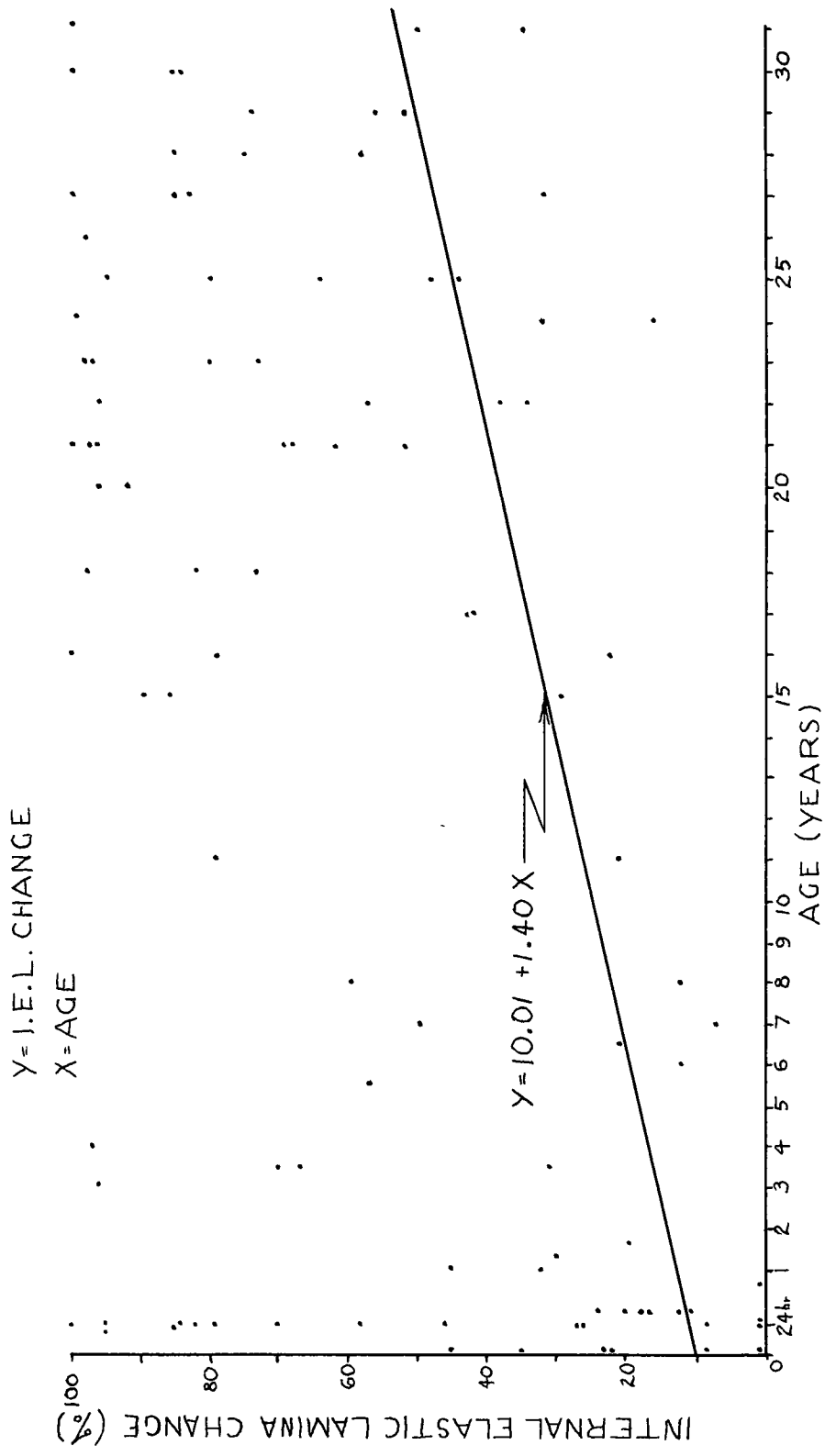

Y = INTIMA
X = I.E.L. CHANGE
Y = 11.75 + 0.35 X
INTIMA (% OF TOTAL WALL)
INTERNAL ELASTIC LAMINA CHANGE (%)
0
10
20
30
40
50
60
70
80
90
100

FIGURE 16


Y = I.E.L. CHANGE
X = AGE
Y = 10.01 + 1.40 X
INTERNAL ELASTIC LAMINA CHANGE (%)
AGE (YEARS)
0
24hr
1
2
3
4
5
6
7
8
9
10
15
20
25
30
20
40
60
80
100

approximation is: Y=10.01+1.40 X. The correlation coefficient r is 0.49 ($p < .01$, error df 99). Although this is a significant correlation, it is not as high as some of the other correlations. This is the result of a low correlation in the younger age groups, as seen in figure 16.

TABLE IX. COMPARISON OF THE AVERAGE % INTERNAL ELASTIC LAMINA CHANGE IN THREE AGE GROUPS. (SEXES COMBINED)

AGE	SAMPLE SIZE	AVERAGE % I.E.L. CHANGE	CALCULATED STUDENT'S T	SIGNIFICANCE $\alpha = .05$
0-1 1-15	28 22	36.44 47.12	t=1.303 df 48	no significant difference $.10<p<.20$
1-15 16-30	22 51	47.12 73.53	t=4.229 df 71	significant difference $p < .001$

It is seen from table IX that there is significant difference in the average percentage internal elastic lamina change only between the 1-15 and 16-30 year age groups. Similar results to table IX are obtained if males and females are compared separately.

Table X compares the differences in the sexes in the three age groups, with respect to the amount of internal elastic lamina change. The only significant difference occurs in the 16 - 30 year age group.

TABLE X. COMPARISON OF THE SEX DIFFERENCES IN THE AVERAGE INTERNAL ELASTIC LAMINA CHANGE IN THREE AGE GROUPS.

AGE	SEX				CALCULATED STUDENT'S T	SIGNIFICANCE $\alpha = .05$
	MALE	SAMPLE SIZE	FEMALE	SAMPLE SIZE		
0-1	42.73	19	23.92	9	t=1.458 df 26	no significant difference .10<p<.20
1-15	54.23	14	34.68	8	t=1.941 df 20	no significant difference .05<p<.10
16-30	79.32	38	56.60	13	t=3.106 df 49	significant difference .001<p<.10

Figure 17 is a graph of the absolute thickness of the media, on the Y axis, compared to the age in years, on the X axis, (101 observations). The equation of the best straight line approximation is: $Y = 23.45 + 7.89\ X$. The correlation coefficient r is 0.69 ($p < .01$, error df 99). This is a highly significant correlation between these two variables.

Figure 18 is a graph of the absolute thickness of the intima, on the Y axis, compared to the absolute thickness of the media, on the X axis. The equation of the best straight line approximation is $Y = -40.46 + 0.10\ X$. The correlation coefficient r is 0.67 ($p < .01$, error df 99), which is highly significant. Since the graph suggests an increasing slope with increasing medial thickness, in order to determine if the intima is growing at a more rapid rate than the media, a logarithmic transformation was done on the data and a regression calculated on this. Using Y = log. intimal thickness, X = log. medial thickness, the equation of the best straight line approximation is: $Y = -5.06 + 1.89\ X$. The correlation coefficient r is 0.76, which

FIGURE 17

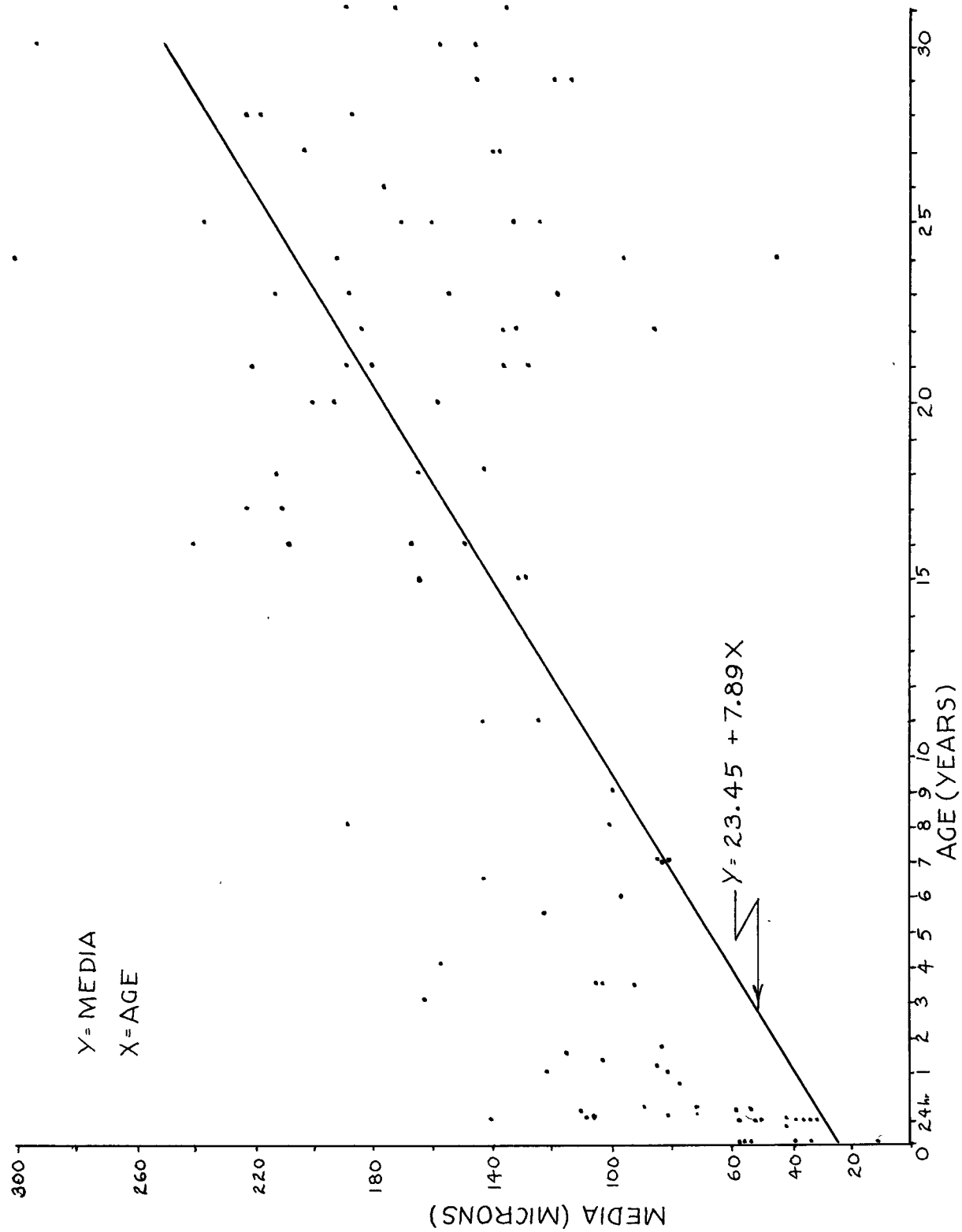
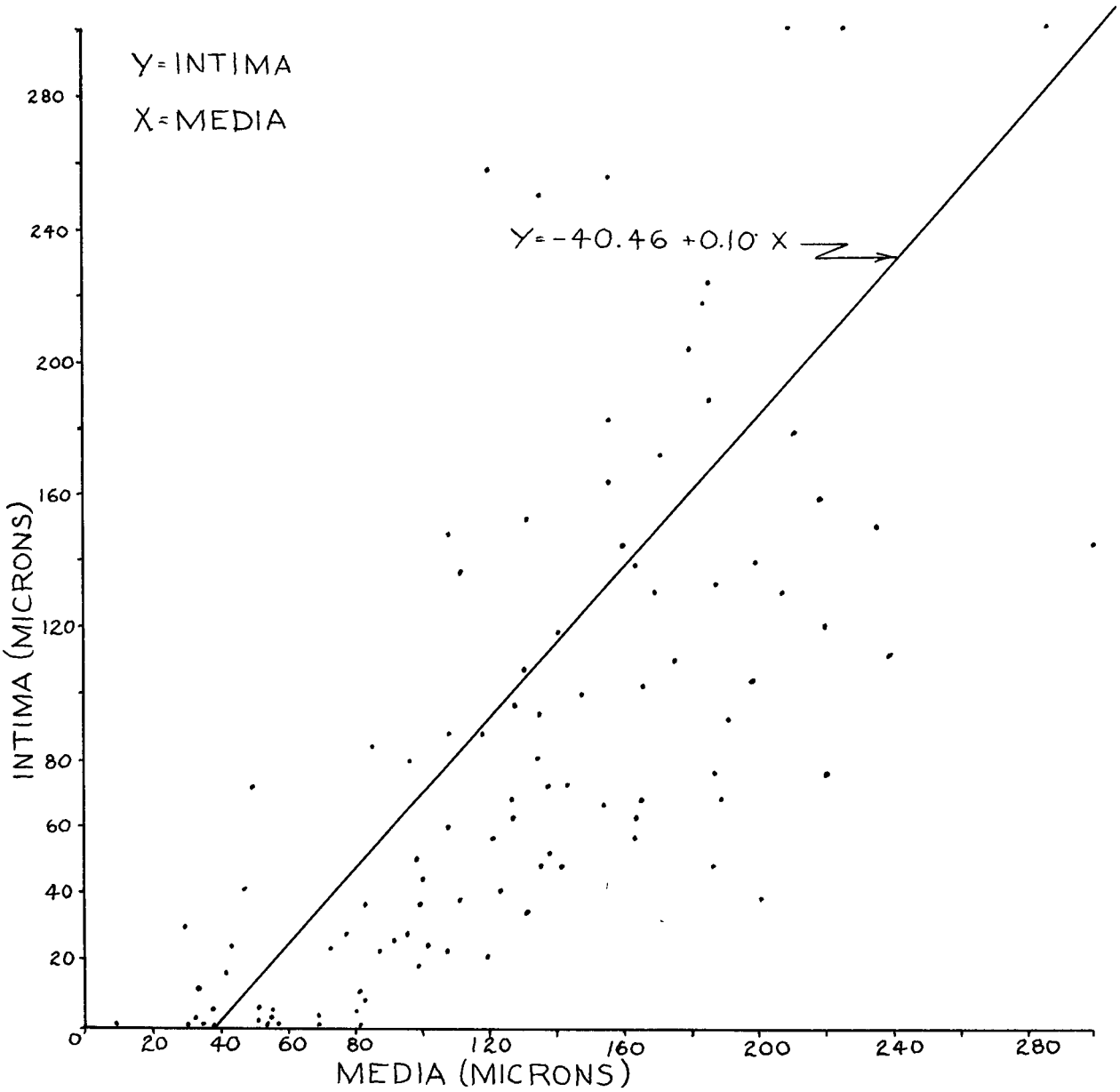

Y= MEDIA
X= AGE
Y= 23.45 + 7.89X
MEDIA (MICRONS)
AGE (YEARS)
0
24h
1
2
3
4
5
6
7
8
9
10
15
20
25
30
20
40
60
100
140
180
220
260
300

FIGURE 18


Y = INTIMA
X = MEDIA
Y = -40.46 + 0.10 X
INTIMA (MICRONS)
MEDIA (MICRONS)
0
20
40
60
80
120
160
200
240
280

is highly significant. Since the slope of a logarithmic transformation of Y on X is greater than 1, this is conclusive evidence that in this set of data the intimal thickness is increasing at a more rapid rate than the medial thickness.

Figure 19 is a graph of the absolute intimal thickness, on the Y axis, compared to the heart weight, on the X axis (83 observations). The equation of the best straight line approximation is: $Y = 20.66 + 0.35 X$. The correlation coefficient r is 0.55 ($p < .01$, error df 81), which is significant.

Figure 20 is a graph of the absolute intimal thickness on the Y axis, compared to the body weight, on the X axis (78 observations). The equation of the best straight line approximation is: $Y - 15.23 + 0.83 X$. The correlation coefficient r is 0.60 ($p < .01$, error df 76), which is highly significant. If a logarithmic transformation is performed on this date, a regression of log. Y on log. X will result in a slope greater than 1. Therefore the rate of intimal growth is faster than the rate of growth for the total body mass.

Figure 21 is a graph of the degree of internal elastic lamina change, on the Y axis, compared to the body mass, on the X axis. It is evident from this graph that there is a very low correlation between these two variables. This is especially true in infants weighing less than twenty-five pounds where there is a great variation in the amount of internal elastic lamina change.

A comparison was made in the 20-30 year age group where

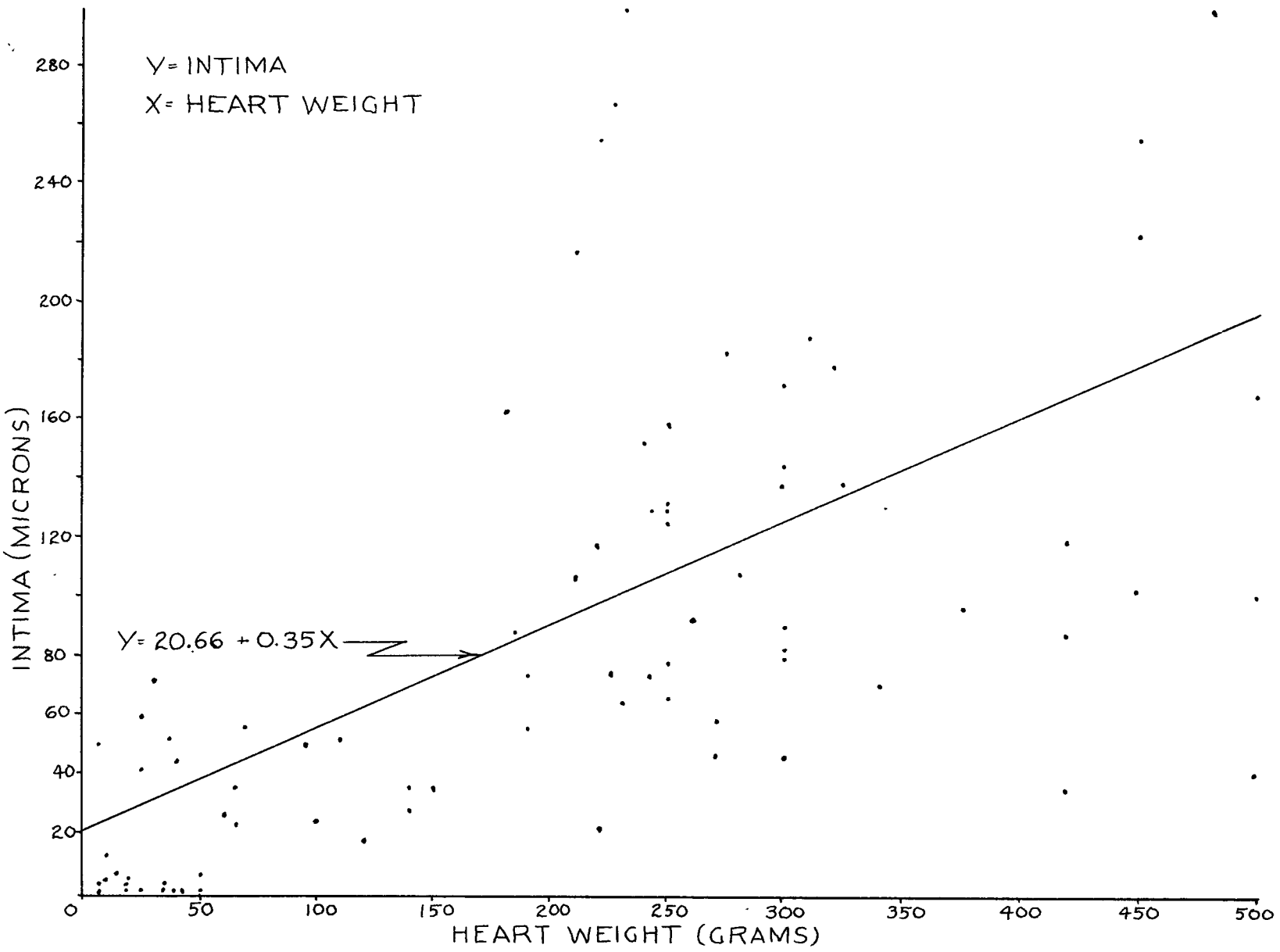

Y= INTIMA
X= HEART WEIGHT
Y= 20.66 + 0.35X
INTIMA (MICRONS)
HEART WEIGHT (GRAMS)
0
20
40
60
80
120
160
200
240
280
50
100
150
200
250
300
350
400
450
500

FIGURE 19

FIGURE 20

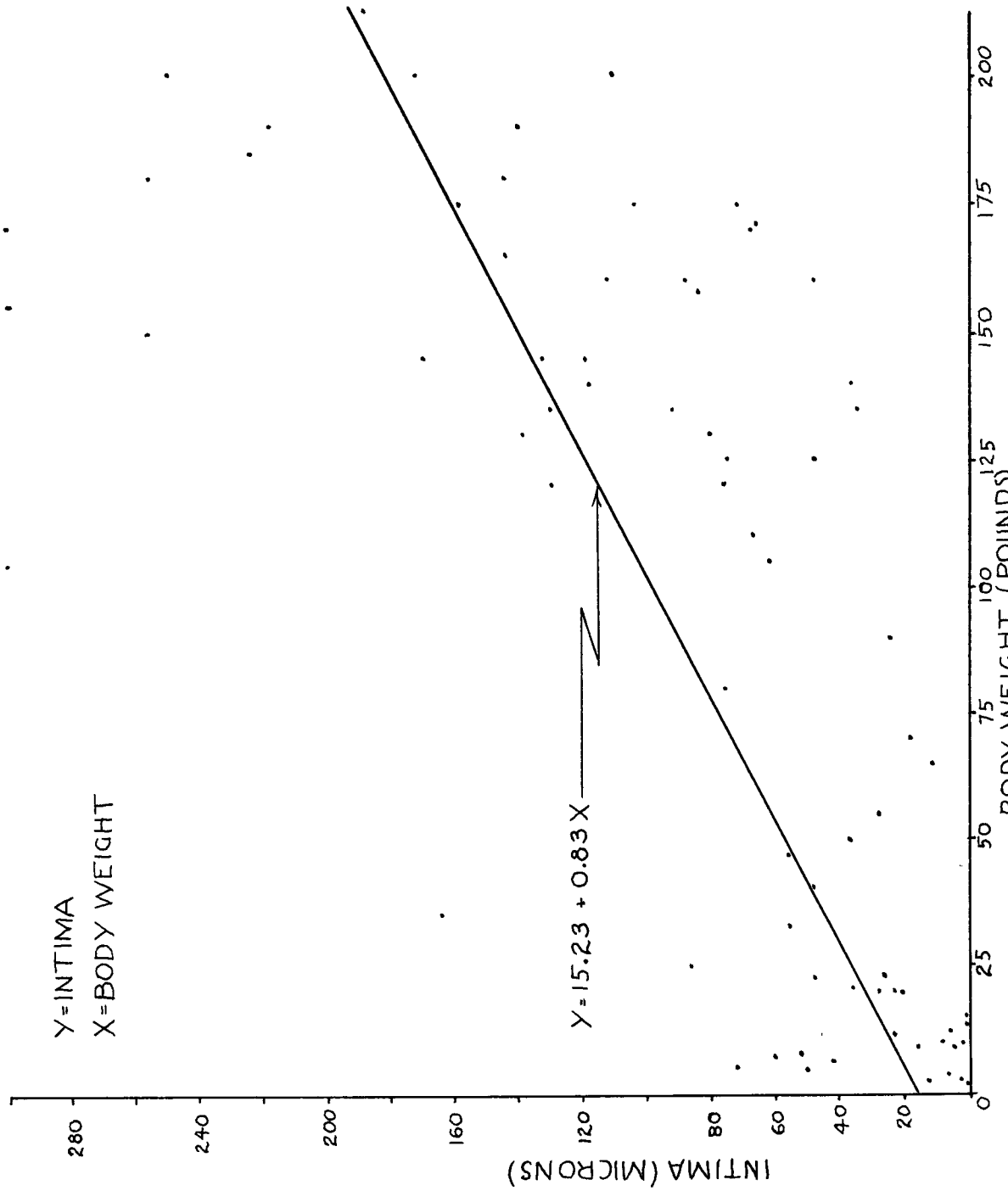
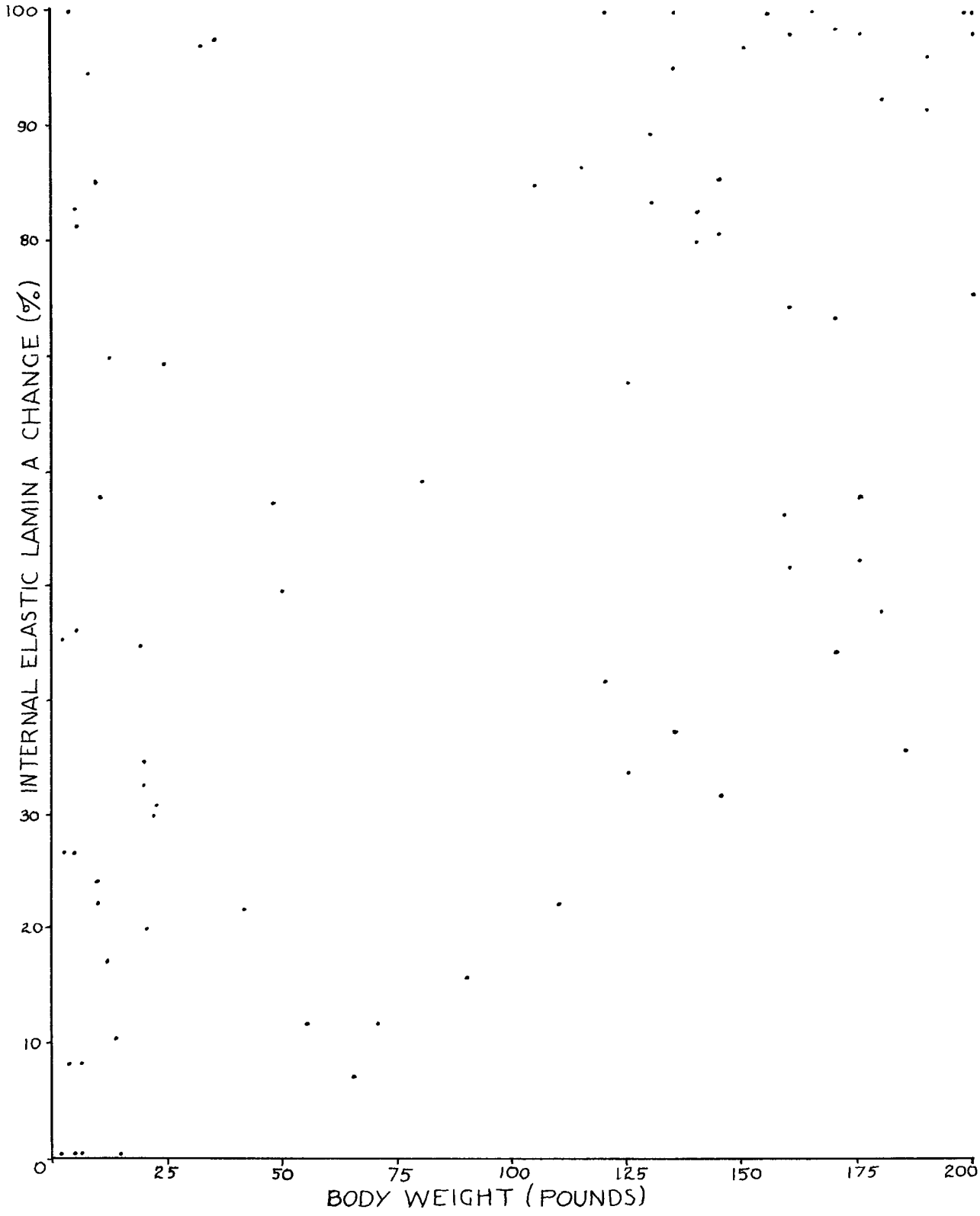

Y=INTIMA
X=BODY WEIGHT
Y=15.23+0.83X
INTIMA (MICRONS)
BODY WEIGHT (POUNDS)
280
240
200
160
120
80
60
40
20
0
25
50
75
100
125
150
175
200

FIGURE 21


100
90
80
30
20
10
0
% INTERNAL ELASTIC LAMIN A CHANGE (%)
25
50
75
100
125
150
175
200
BODY WEIGHT (POUNDS)

the 20 specimens with the greatest hyperplasia were compared with the 20 specimens with the least hyperplasia to endeavour to find a correlation. The same correlations with regard to sex, heart weight, and body weight, that have been discussed were found. Thirty percent of the group with the greatest hyperplasia died from chronic disease compared to only ten percent of the group with the least hyperplasia. This difference is not statistically significant and could be just due to chance.

CONCLUSIONS AND DISCUSSION:

From figures 10-12 and tables V-VII it is shown that the intimal layer of the coronary artery correlates very closely with age in the 0-30 year range studied. Although the presentation of data suggests a direct relationship, it is understood that age itself is not necessarily a factor responsible for intimal growth but that there are one or many factors which are operating either continuously or intermittently throughout this age period to stimulate intimal hyperplasia.

Contrary to Dock's (37) and Lober's (91) findings, no significant difference in the intimal thickness in males and females was found under the age of 15 years. But, there was shown to be a difference in the age group from 16-30 years where the intima in the male is greater than that of the female. This is shown in table VII, and suggests that some difference operating at and after puberty has an influence either on stimulating the male or inhibiting the female intimal growth. Apart from the dietary and activity differences in the two sexes, the hormonal difference has been suggested as an influencing factor. Do the estrogenic hormones, with a proliferative effect on epithelial tissue but not so much on mesenchymal tissue, have a relative retarding effect on the intimal growth? Actually, little is known about the influence of sex hormones on human connective tissue. The estrogens will increase the amount of mucopolysaccharides, particularly hyaluronic acid content, in all connective tissue.

But, Testosterone, as well as stimulating muscle growth and mesenchymal tissue, also increases mucopolysaccharides sensitive to hyaluronidase (10). Since connective tissue changes are often manifestations of hyper or hypofunction of the hormones, it is conceivable that a sudden change in the hormonal content, which occurs at puberty, could upset the metabolism of the arterial wall. Lober (91) claims that there is a sex difference before puberty, but that after the age of ten years the rate of progression of male and female intimae are the same, and for this reason he states that the hormonal differences are not fundamental. In his study he investigated also the post menopausal period and found that the hormonal change here also did not appreciably effect the intimal thickness.

From figures 13 - 15 it is shown that the intimal thickness is directly proportional to the amount of destruction of the internal elastic lamina, using both the absolute and relative thickness of the intima. If we accept the theory that the internal elastic lamina is the site of the first injury, and that the intima is a biological expression of regeneration in response to the degeneration of the internal elastic lamina, this correlation between intimal hyperplasia and the amount of destruction of the internal elastic lamina is expected. Also, it has been shown experimentally that after injury to the internal elastic lamina (or arterial wall) by various chemical and physical agents there follows a reparative intimal proliferation (15, 29, 67, 79, 80, 118, 140, 147, 160). Therefore, this is probably a true cause

and effect correlation: that is, internal elastic lamina change is the primary event in the process of intimal hyperplasia. Supporting this statement is the fact that in this study there is better correlation of the amount of intimal thickness with the amount of internal elastic lamina change than the correlation between the intimal thickness and the age.

Similarly, there would be expected a relationship between age and the amount of internal elastic lamina destruction. Figure 16 and table IX demonstrate a statistically significant increase in the change from the normal of the internal elastic lamina with increasing age from one year to 30 years. In the age group 0-12 months this is not so in the present study. Here 8/26 of the babies dying in the first two weeks showed internal elastic lamina change greater than 50% which produces a non-significant difference for this age group. This is carried over somewhat into the 1-15 year age period (see table VIII). This is a reflection of the type of specimen used. In the age group over five years most of the patients could be considered normal from the fact that they died from traumatic or acute causes. However, many of the infants died from disease processes which are present from, or even before, birth. Disturbances in this period affect many tissues including possibly the elastic tissue. These metabolic upsets may cause large amounts of internal elastic lamina destruction which, in other people, may take up to thirty years to produce. This suggests that perhaps some metabolic poison is present in high concentrations in these diseased

infants, which may injure elastic tissue rapidly here, but takes much longer in relatively healthy individuals. Although this metabolic factor is unknown it may act similarly to known metabolic injurious factors such as the lathyrus factor, B-aminoproprionitrile, which attacks connective tissue by blocking the production of collagen from the fibroblast. This is due to a disturbance in mucopolysaccharide metabolism, possibly as a result of depolymerization of the ground substance (chondroitin sulfuric acid) which can cause an "injury" to the arterial wall (141).

In this study there was found a sex difference in the amount of internal elastic lamina destruction in the age group 16-30 years (males greater than females) but not in the younger groups (table X). This is more evidence supporting a hormonal influence at the pubertal period.

Figure 17 confirms earlier studies (91) that the growth of the media continues with age, plateauing off at the 20-30 year age period, suggesting a pattern similar to general body growth.

When comparing intimal thickness with medial thickness it is shown that there is a certain parallelism between the two, but, it a logarithmic transformation is done, it is shown that the intima increases at a more rapid rate than the media. (The equation of the slope b for log. Y on log. X is: $Y = 1.89\ X$.) Following on this, if the media conforms to generaly body growth, then the intima is growing faster than general body growth, and therefore is an abnormal or non-physiological process.

Similarly, Figures 19 and 20 show the relationship of intimal growth to heart mass and body mass respectively. Employing a logarithmic transformation on these graphs also proves a faster rate of growth of the intima than the heart or body mass. In this study it is not possible to derive similar conclusions, as the above, for the internal elastic lamina (figure 21).

In order to clarify some of the findings of this paper, it would be necessary to further investigate the infants by obtaining a large sample of normal specimens to compare with ones with disease processes. Since invivo measurements are, at present, impossible, and because of the nature of infant mortality, this would require a long period of specimen collection. Also, since some internal elastic lamina change is seen at birth, perhaps some work should be done on the intra-uterine period of growth.

From experimental work on injurious agents, and suggestions from studies like the present one, that perhaps some metabolic agent is acting along with, or instead of, a strictly mechanical factor of etiology, further search for the possible agent(s) must be done. There are many injurious agents which have been used experimentally in animals, but somehow these agents must be tied into human intimal hyperplasia. Perhaps some metabolic factor from the diseased infants, which suggested such a material, could be used to promote animal intimal hyperplasia, as the reverse is not possible. A careful study of a series of cases might give clues to such metabolic conditions and their possible

effects on the internal elastic lamina. In the present study the diseases in the infant group were very variable (table III) but, with a large percentage of respiratory problems.

Since the precise nature of the injurious factors is unknown the question as to the possibility of preventing or retarding intimal hyperplasia cannot yet be answered.

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