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Plasma Protein Insudation as an Index of Early Coronary Atherogenesis

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Two hundred ninety-nine paraffin-embedded coronary artery blocks from 68 autopsy cases were serially sectioned. The blocks were selected to provide a range from normal through various stages of atherosclerosis, and sections were examined with the indirect immunofluorescence technique for intramural distribution of plasma albumin, fibrinogen, and immunoglobulin γ (IgG). Cryostat-sections of 44 blocks from 22 of the same cases were examined with the same technique for distribution of apolipoprotein B. Alteration of protein insudation in the artery wall was a sensitive index of coronary atherogenesis. The sequence in which these proteins were involved in the initiation and development of early atherosclerotic lesions was analyzed by determining the average relative intimal thickness and relative lumen size that was associated with the first occurrence of altered insudation of each of these proteins. Results indicate that changed plasma albumin insudation is the earliest sign of a focal intimal lesion, and increasing albumin insudation shows the strongest association with intimal plaque growth. The other proteins tested showed altered insudation, in the order IgG, fibrinogen, apolipoprotein B. The results indicate that a progressive increase in permeability of the coronary artery endothelium occurs in the early stages of atherogenesis. Patterns of IgG localization provide evidence of both early systemic and subsequent local immune reactions being involved in atherogenesis. Altered albumin and apolipoprotein B insudation levels have stronger correlation coefficients with relative intimal thickness and relative lumen size than do those IgG and fibrinogen. The extremely high correlation coefficients shown by albumin emphasizes the importance of edema in determining plaque size and lumen stenosis. (Am J Pathol 1993, 143:496–506)

Atherosclerosis research is typically concerned with visible established plaques which can show a variety of histopathological changes, including foam cell and lipid accumulation, fibrosis, calcification, smooth muscle cell proliferation, inflammation, neovascularization, necrosis, hemorrhage, and edema.[1–3] Examination of established plaques cannot provide readily interpretable information on the initiation of atherosclerotic lesions, because of the episodic, relapsing character of the disease, which entails periods of dormancy, activity, and repair.[2–5]

Changes in the insudation of blood components have been used to investigate the effect of blood pressure on the passage of plasma into canine aortic wall[6] and the involvement of immunoglobulins in the development of atherosclerosis in the aorta.[7–8] However, few reports relate the infiltration of blood components into human coronary artery walls to the initiation of atherosclerotic lesions.

The present study was designed to use indirect immunofluorescence techniques,[9] in conjunction with high-resolution confocal microscopy, to examine the distribution of various plasma proteins in human coronary artery walls. We have analysed their earliest changes in relation to relative intima-to-wall thickness (I/W) and relative lumen-to-total vessel size (L/T), so as to examine the role that insudated blood components may play in the initiation of atherosclerosis. In addition, effects of more severe degrees of plasma protein insudation, or of *in situ* synthesis (in the case of IgG), were examined by regression analysis with respect to I/W and L/T.

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Materials and Methods

Sixty-eight human hearts (43 male and 25 female, with ages from 19 to 89) were obtained at autopsy from the Pathology Department of Royal Canberra Hospital and from the School of Pathology, University of New South Wales, Sydney. The causes of death of these subjects comprised acute myocardial infarction, other cardiovascular diseases, infectious diseases, cancer, and suicide or accident. Coronary arteries from these cases were cannulated and perfused with formalin at 100 mm Hg. Sections were cut from 299 paraffin-embedded blocks and measured with the MD-1 Microscope Digitizer system (Minnesota Datametrics, St. Paul, MN) for calculation of the I/W and the L/T. Then paraffin-embedded serial sections from the same blocks were examined for distribution of plasma albumin, fibrinogen, and IgG by the indirect immunofluorescence technique.[10–12] Full details concerning origin of the coronary arteries, tissue fixation, methods of examination, and morphometric techniques used have been described previously.[13]

In addition, 44 coronary artery blocks, stored in 100% glycerol from the same cases, were placed in deionized water, decalcified with 10% ethylenediaminetetraacetic acid (EDTA) and snap-frozen in n-hexane (95%) at −70 C, supported in Bright CRYO-M-BED Embedding Compound (Bright Instrument Co., Ltd, England), and cryostat-sectioned at 8 μm. Sections were mounted on poly-L-lysine-coated slides (Sigma Chemical Co., St. Louis, MO) and stored at −25 C prior to staining for the detection of apolipoprotein B.

Detection of Apolipoprotein B

Frozen sections were air-dried at room temperature for 20 minutes rinsed three times for five minutes each time in water followed by three 5 minute immersions in phosphate-buffered saline (PBS) containing 0.5% Tween 20. Rabbit anti-human apolipoprotein B antibody or normal rabbit serum was added to the sections in a humidified chamber for 30 minutes. After three 5 minute washes with PBS, sections were incubated with the second antibody at 37 C for 30 minutes, washed three times for 5 minutes each time with PBS, and mounted in PBS and glycerol (1:9) at pH 8.6 under glass coverslips.

Classification of Immunoreactivity Grades

Albumin

Grade 0: Diffuse albumin immunofluorescence normally present throughout the intima and media.
Grade 1: Albumin-deficient area present in the thickened intima surrounded by a zone of albumin fluorescence brighter than the grade 0 base level (Figure 1).
Grade 2: As in grade 1, but with bright areas of albumin immunofluorescence present within plaques also.
Grade 3: Uniform strong albumin-specific immunofluorescence throughout in large atherosclerotic plaques. The pattern is virtually identical to that shown by fibrinogen grade 3 in Figure 3.

IgG

Grade 0: No specific immunofluorescence.
Grade 1: Immunoglobulin-positive elongated and small rounded profiles or small well delineated positive areas present in the intima.
Grade 2: Immunoglobulin-positive cells, up to 10 cells per × 20 objective field, distributed separately or in small groups in the thickened intima.
Grade 3: Immunoglobulin-positive cells, more than 10 cells per × 20 objective field, present within plaques (Figure 2).

Fibrinogen

Grade 0: Negative
Grade 1: Diffuse fibrinogen-positive band in subendothelial region.
Grade 2: Fibrinogen immunofluorescence surrounding intimal microvessels.
Grade 3: Includes three subpatterns:
A. Uniform fibrinogen-specific reactivity throughout major part of the plaque (Figure 3).
B. Fibrinogen-specific thrombi on the surface of plaques or else incorporated into plaques by fibrous organization.
C. Fibrinogen-specific thrombus occluding artery lumen.

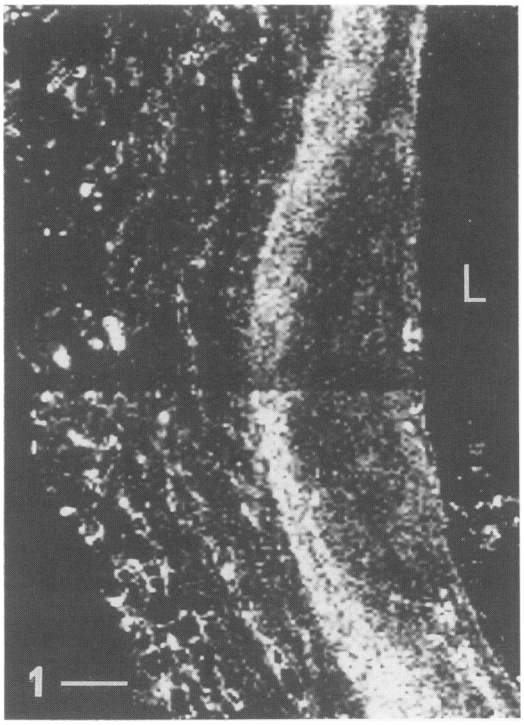

Figure 1. *Albumin-specific immunoreactivity of grade 1 with an albumin-deficient area in the coronary intima. Composite computer figure from two frames. L: artery lumen. First antibody: rabbit anti-human albumin. Second antibody: fluorescein isothiocyanate (FITC)-conjugated sheep anti-rabbit immunoglobulin. Confocal fluorescence microscopy. Scale bar = 50 μm.*

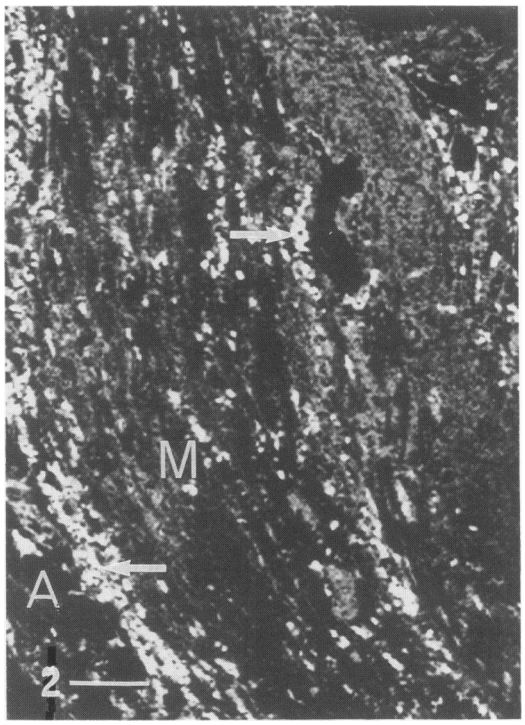

Figure 2. *IgG positive cells (arrows) distributed in adventitia* **(A)**, *the base of an atherosclerotic plaque and the related media* **(M)** *of a coronary artery. First antibody: rabbit anti-human IgG. Second antibody: FITC-conjugated sheep anti-rabbit immunoglobulin. Confocal fluorescence microscopy. Scale bar = 100 μm.*

Apolipoprotein B (apo-B)

Grade 0: No specific immunofluorescence in the intima.

Grade 1: Specific fluorescence restricted to subendothelial regions (Figure 4).

Grade 2: Discrete area, or areas, of apo-B immunofluorescence within the deeper zones of the intima.

Grade 3: Major part of plaques show specific fluorescence (Figure 5).

Morphometric Techniques

The total cross-sectional area (T), medial area (M), intimal area (I), wall area (W), and adjusted lumen area (L) were derived from measurements made with the MD-1 Microscope Digitizer system (Minnesota Datametrics) as described previously.[12] I/W is the ratio of intima area to wall area and reflects the size of plaques. L/T is the ratio of luminal area to total cross-sectional area and is a measure of stenosis.

Statistical Methods

Correlation coefficients between immunoreactivity grades and I/W or L/T were calculated with the Macintosh computer software package Cricket Graph. The significance of differences between average ratios for each grade was calculated by 2-tailed Student's *t*-test and $P \leq 0.05$ was recognized as a significant difference occurring between two groups.

Results

Albumin

In normal arteries, specific albumin immunofluorescence was found in the intima, media and adventitia. Albumin-deficient zones surrounded by rims of enhanced albumin fluorescence developed in thickened intimas (Figure 1). With frank atherosclerotic lesion formation, in addition to albumin insudating from the artery lumen, zones of albumin immunofluorescence were found surrounding new vessels that had invaded the artery wall. The intensity of albumin fluorescence was strongly associated with the severity of the atherosclerotic process, as judged by the richness of neovascularization, lipid deposition,

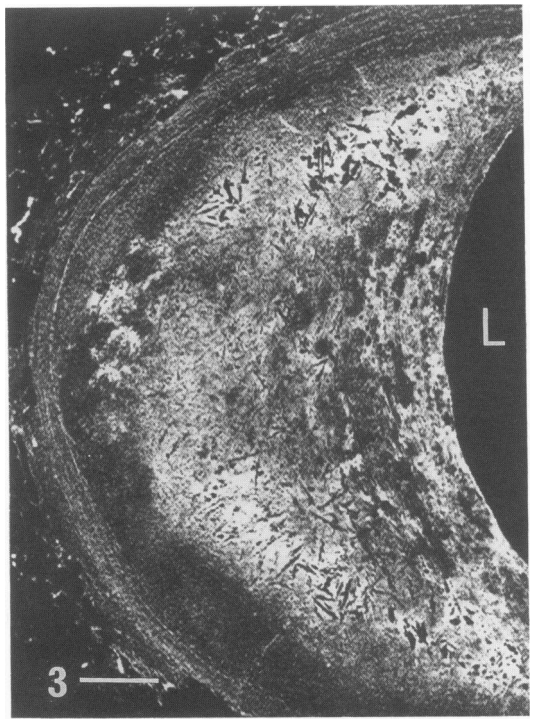

Figure 3. *Coronary atherosclerotic plaque showing strong fibrinogen-specific immunofluorescence with similar pattern to that of albumin distribution.* L: *artery lumen. First antibody: rabbit anti-human fibrinogen. Second antibody: FITC-conjugated sheep anti-rabbit immunoglobulin. Confocal fluorescence microscopy. Scale bar = 100 μm.*

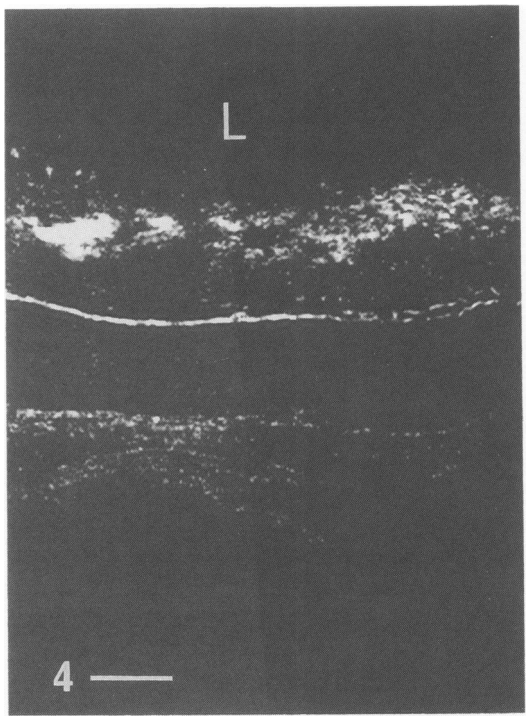

Figure 4. *Apo-B immunoreactivity in the superficial regions of a slightly thickened coronary intima. Note bright autofluorescence of internal elastic lamina (IEL).* L: *artery lumen. First antibody: rabbit anti-human apo-B. Second antibody: FITC-conjugated sheep anti-rabbit immunoglobulin. Confocal fluorescence microscopy. Scale bar = 100 μm.*

intramural hemorrhage, and inflammatory cell infiltration. Whereas intramural albumin was present in all sections examined, altered patterns of insudation occurred in 85% (255/299) of them.

Examination of the sections with a normal pattern of albumin distribution (grade 0) revealed that 90.9% (40/44) had thin intimas with I/W < 0.4 (Figure 6). Arteries with thickened intimas (I/W > 0.4) showed the grade 0 pattern of albumin distribution in only 1.57% (4/255) of sections (Figure 6).

Albumin immunoreactivity grades were negatively correlated with relative lumen size ($r = 0.773$). The average L/T decreased significantly as the albumin reactivity grades increased ($P < 0.001$).

IgG

No IgG was identified in either the intima or the media of normal coronary arteries. A few IgG-positive cells were at times observed in the adventitia. Altogether, 58% (174/299) of the sections were IgG positive.

IgG-specific elongated and rounded fluorescent profiles were often present in thickened fibroelastic or fibromuscular intimas. Intracellular IgG was present in atherosclerotic plaques and the adjacent attenuated media and the adventitia (Figure 2). Advanced plaques at times had diffuse strong IgG-specific fluorescence in their lipid-rich cores.

The IgG immunofluorescence grade of each of the sections was plotted against the I/W (Figure 7) and also against the L/T. Arteries showing IgG grade 0 and 1 were distributed over wide ranges of I/W and L/T ratios. The I/W ratio was significantly different between immunoreactivity grade 0 and grade 1 ($P < 0.001$), whereas the L/T ratio showed no significant differences between them.

Fibrinogen

It was not possible to distinguish immunologically between fibrinogen and fibrin. No fibrinogen was detected in normal coronary walls. It first appeared as a subendothelial band within thickened fibromuscular intimas. Focal specifically stained areas were found where groups of new vessels or cellular infiltrates were present, usually in plaque shoulder regions. The largest deposits were in advanced plaques with rich neovascularization and/or severe

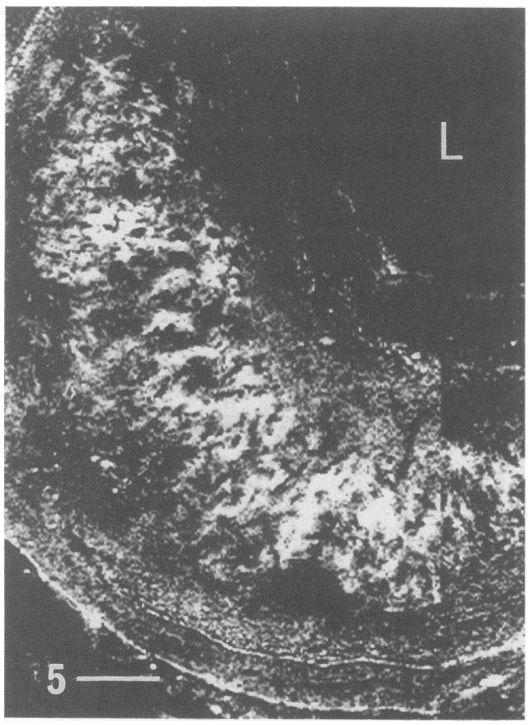

Figure 5. *Strong apo-B immunoreactivity in atherosclerotic plaque. L: artery lumen. First antibody: rabbit anti-human apo-B. Second antibody: FITC-conjugated sheep anti-rabbit immunoglobulin. Confocal fluorescence microscopy. Scale bar = 100 μm.*

inflammatory cell infiltrates (Figure 3). At times, specific green immunofluorescence was mixed with the yellow autofluorescence of oxidized lipid in plaques full of cholesterol and/or lipid-rich gruel. Adherent fibrin thrombi were occasionally observed on the surface of plaques, at times completely occluding the very narrow lumen.

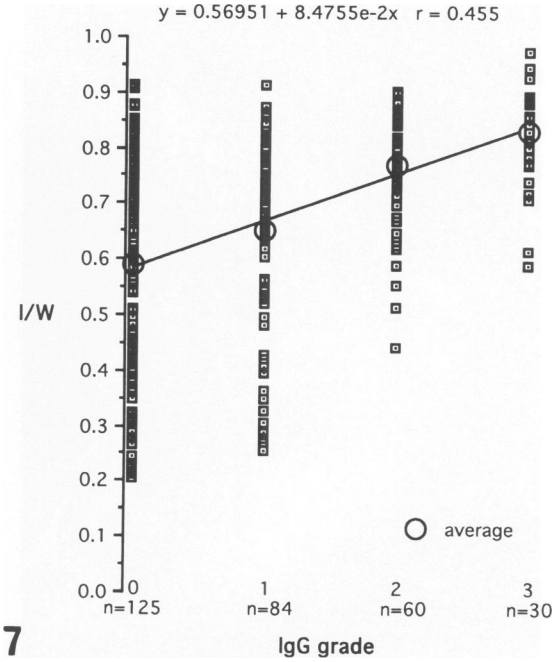

Figure 7. *IgG immunoreactivity grades versus I/W.*

Fibrinogen grades were plotted versus the I/W (Figure 8) and also versus the L/T. Fibrinogen immunoreactivity was present in 28% of sites (84/299). Although fibrinogen immunoreactivity increased

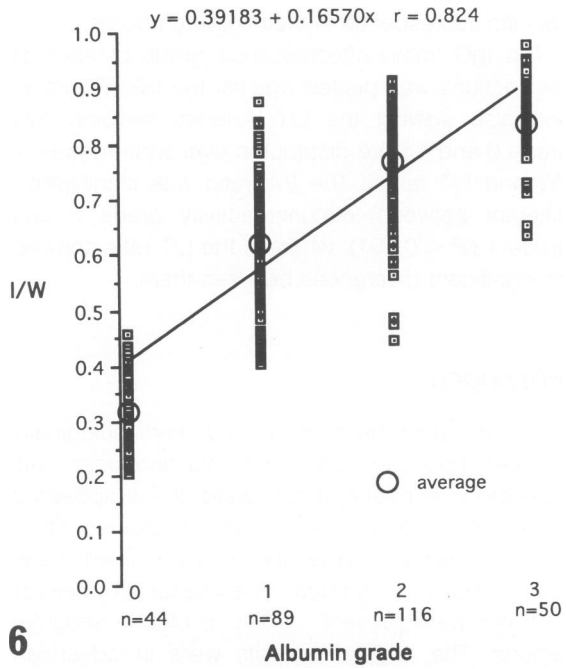

Figure 6. *Albumin immunoreactivity grades versus I/W.*

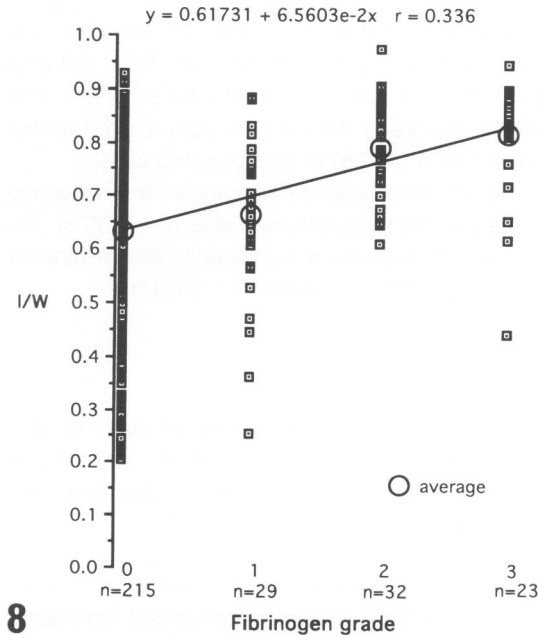

Figure 8. *Fibrinogen reactivity grades versus I/W.*

with I/W ratio (r = 0.336) and decreased with L/T ratio (r = 0.346), there were no significant differences of these ratios between grades 0 and 1.

Apolipoprotein B

These studies were performed on cryostat sections of the coronary arteries. Apo-B first appeared in immediately subendothelial regions when the thickness of the intima exceeded that of the media (Figure 4). As intimal thickness increased, apo-B extended to the base of the intima, where it was closely associated with elastic tissue fibers and the internal elastic lamina. In advanced plaques, apo-B was related to collagen of the fibrous caps and to elastic fibers within the plaques. The richest deposits occurred in the lipid cores of atheromatous plaques (Figure 5). Calcified areas within plaques were often positive for apo-B.

Altogether 73% (32/44) of sites examined were positive for apo-B. Apo-B immunofluorescence grades are plotted against I/W in Figure 9. Apo-B grades increased with I/W (r = 0.724). Only one of the arteries with I/W < 0.6 was positive for apo-B staining (Grade 1) and all but one of the positive arteries had I/W > 0.6. Almost all of the arteries with I/W > 0.6 showed apo-B specific staining (31/34, 91.2%).

Apo-B immunofluorescence grades were also plotted against L/T. There is a negative correlation between apo-B grades and L/T ratios (r = 0.647).

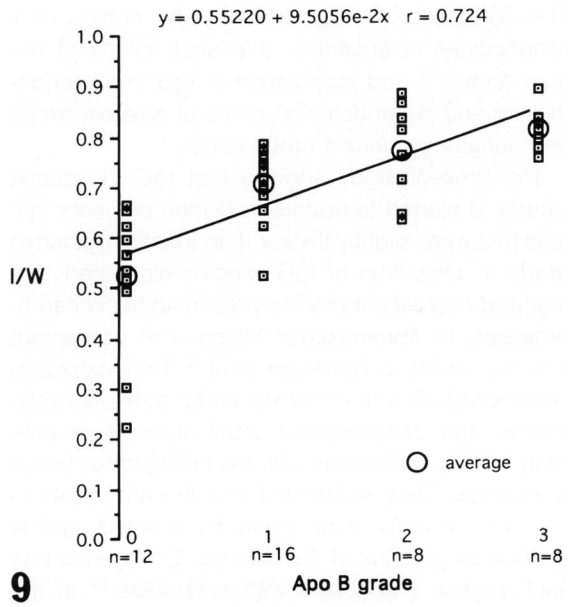

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Figure 9. *Apo-B reactivity grades versus I/W.*

Table 1. *Statistical Difference Between Grade 1 and Grade 0 of Immunoreactivity*

Immunoreactivity	I/W	L/T
Albumin	+*	+
Apo-B	+	+
IgG	+	–†
Fibrinogen	–	–

* +, significant difference.
† –, non-significant difference.

The differences of I/W and L/T between apo-B grade 0 and grade 1 are both significant ($P < 0.01$).

Discussion

The I/W ratio gives the relative intimal thickness and the L/T ratio gives the relative lumen size. They are the standard indices of coronary lesions used in this study. They each relate to a different aspect of the atherosclerotic process. The I/W ratio gives an indication of the activity of the disease in producing more intimal tissue, implying increasing lipid deposition and increasing fibrosis.[14] It is not, however, a direct measure of stenosis, or reduction of the arterial lumen, which is given by L/T, the ratio of luminal area to total cross-sectional area of the artery. Our results showed that these ratios for both albumin and apo-B grade 1 immunoreactivity are significantly different from those for corresponding grade 0. Albumin and apo-B are both normally present in the blood and are continuously available to enter atherosclerotic lesions. Hence, changes sufficient to alter the albumin pattern were present in 85% of the sites, and changes severe enough to alter apo-B insudation were present at 73% of the sites.

IgG and fibrinogen immunoreactivity grade 1 sites are not significantly different from grade 0 sites, in terms of the L/T ratio (Table 1). The sections showing IgG and fibrinogen immunoreactivity grade 0 varied in a wide range of L/T ratios. This demonstrated that atherosclerotic disease is a kind of chronic disease, and some processes associated with atherogenesis are not continuous but wax and wane and start and stop.[2,4–5] In terms of I/W there was a significant difference in IgG immunoreactivity grade 1 and 0, but this was not the case for fibrinogen. Fibrinogen has long been recognized as a very important factor in the formation of atherosclerotic lesions.[15–18] The results herein show that the involvement of fibrinogen is on a short time scale, as it is rapidly organized and replaced by fibrous scar tissue.[19] The average I/W or L/T of grade 0 for this component will be profoundly altered in arteries

in which such processes have occurred. Hence, comparison of the highest I/W or lowest L/T for the various components at grade 0 is unlikely to give useful information on intimal component changes in the early stages of atherosclerotic lesions. Grade 1 is always classified as the earliest positive alteration in immunoreactivity, which, in the case of albumin, is a change in distribution pattern from the one that is normally present.[6] Therefore, the sequence of events involved in the early atherosclerotic lesions has been analysed in terms of the average I/W and L/T which is associated with the appearance of the test substance immunoreactivity grade 1 (Figure 10).

Altered albumin insudation was the first sign of focal intimal lesions, with the average I/W for albumin immunoreactivity grade 1 being the lowest for all the components tested. Albumin is a low molecular weight protein that constitutes the largest proportion of the normal plasma proteins and has been used as a tracer for plasma movement in a number of investigations.[6,20,21] The presence of albumin in the walls of human aortas and coronary arteries has been demonstrated by extraction[22] and by histological observations.[23] The present work confirmed the presence of albumin in the walls of all the human coronary arteries examined and found that altered patterns of albumin distribution were very strongly correlated with intimal thickness. When the I/W ratio exceeded 0.4, an altered intimal pattern of albumin-rich and -deficient zones appeared which was very similar to the patterns of abnormal albumin distribution described by Adams, Bayliss and Morgan.[24] They injected trypan blue solution into cholesterol-fed rabbits and found that the normal intima surrounding raised lesions in the aorta was strongly stained by trypan blue complexed with albumin. They speculated that such stained zones were the main sites of atherosclerotic lesion growth and progression. In general, blood-derived components within the intima are considered to have perfused physiologically from the artery lumen[23] and to have infiltrated from the *vasa vasorum* in the adventitia. Increased endothelial permeability is proposed as a major factor in the initiation and localization of atherosclerotic plaques.[1] Bell and colleagues[25] studied regions of increased passage of both Evans Blue and ^{131}I-albumin into the aorta of swine. The 'blue areas' were regions of increased entry of dye and of albumin into the arterial intima and were interpreted as being regions of endothelial damage. Such changes result in the intima absorbing more substances, including albumin.[5] The albumin-deficient zones in the intima in such instances may be due to the blood-derived material not reaching the deeper layers of the intima.[26] In addition, intimal damage may result in increased synthesis of polysaccharides[27-29] which would retain water in the middle of the intima and exclude albumin to the edges of the lesions.[30] These two processes in combination would result in the pattern of altered albumin distribution initially observed.

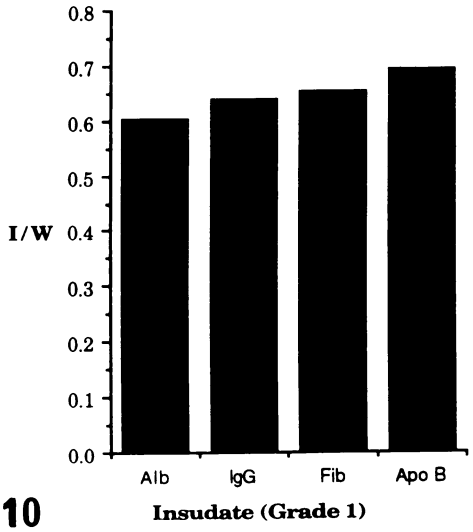

Figure 10. *Average I/W at which the substances tested are present at immunoreactivity grade 1.*

A correlation between lymphocytic infiltration in the adventitia of arteries and the severity of atherosclerosis was identified by Schwartz and Mitchell.[31] This suggested the involvement of immunoreactions in atherogenesis which has subsequently been examined as a possible cause for this disease.[7,32] This suggestion is supported by the finding of T lymphocytes in eccentric, thickened intima of human aortas,[33] and localization of IgG in the endothelium and subendothelial intima of atherosclerotic and nonatherosclerotic rabbit aortas.[8]

The present study showed that IgG insudation (grade 1) started to appear in human coronary intimas that were slightly thicker than those for albumin grade 1. Detection of IgG-specific elongated and rounded fluorescent profiles present in thickened fibroelastic or fibromuscular intima is in agreement with the results of Hollander et al.[22] They extracted immunoglobulins from human aortic atherosclerotic intimas and demonstrated small amounts of antibody closely associated with the collagenous tissue of plaques. They suggested that the antibodies in the atherosclerotic plaque may be directed against the fibrous proteins of the arteries. Collagen is one such protein that reacts with antibodies.[34] In the present observations, this kind of antibody localiza-

tion was identified not only in the fibrous caps of atherosclerotic plaques, but was also within the fibroelastic-thickened intimas, indicating that antibody reactions may start in the early stages of atherogenesis. There are, in addition, other connective tissue proteins that have been demonstrated as having antigenic properties capable of inducing specific antibodies.[35] The observed antibodies may be binding to arterial components closely associated with collagen, such as lipoproteins or acid mucopolysaccharides.[36–37] An alternative explanation is that the antibodies are within deposits of antibody-antigen complexes. Such immune complexes have been found in arteries with inflammatory reactions produced by foreign proteins.[38–39]

In the present study, intimal thickness was significantly increased in arteries that showed insudation of immunoglobulin. It is possible, therefore, that some form of antibody-mediated systemic reaction is playing a role in the early stages of atherogenesis.

IgG-containing cells were present only in the adventitia and within atherosclerotic plaques, confirming other workers' observations.[40–41] The IgG-positive cells in the present study were always located in areas of inflammatory cell infiltration. The density of IgG-positive cells closely correlated with increase of intimal thickness and decrease of lumen size, suggesting that a locally mediated immune reaction is involved in the developing atherosclerotic process. IgG-positive cells were often found surrounding microvessels in the artery wall, and the numbers of positive cells correlated strongly with the degree of vascularization. It would appear that the microvessels in the artery walls actually provide the antibody-producing cells in the plaques.

In a few cases, IgG-containing cells were found only in the subendothelial regions overlying atherosclerotic plaques. Whether they were produced following endothelial damage and increased endothelial permeability is not known, but antigens are present in endothelial cells that can attract and stimulate antibody-producing cells.[42]

The diffuse deposition of IgG occurred in the lipid-rich cores, which may accumulate a considerable quantity of antigens. Ceroid is one of the antigens that reacts with IgG in these areas,[43] and C-reactive protein may be another one.[44] Residual antibodies bound or deposited before the lipid cores were formed should be considered as another possible source of immunoglobulins in the lipid cores. The physical conditions that lead to the pooling of lipid and debris within the arterial intima may also operate in a purely passive way in the cases of other large molecules, such as immunoglobulins. In addition, lipoproteins are the most antigenic components of the blood[45] and in the lipid cores may very possibly react with antibodies.

Finally, herpes simplex virus and cytomegalovirus nucleic acids have been demonstrated in atheromatous plaques of human coronary arteries.[46] It has been suggested that such viruses may be the antigens that stimulate immunoglobulin production. The percentage of sites identified as IgG-positive was surprisingly high (58.2%) and stresses the possibility of the widespread occurrence of immune processes occurring within the coronary arteries of the population in general.

In terms of the relative intimal thickness, fibrinogen insudation grade 1 appears at apparently a later stage than IgG grade 1 (Figure 10), but their average I/W ratios show no statistically significant difference. The presence of fibrinogen is often associated with IgG insudation in corresponding areas of serial sections. This supports the idea that some form of infection or immunoreaction may be occurring in the endothelium or within the intima that greatly increases endothelial permeability, leading to fibrin deposition.[4] For relative lumen size, fibrinogen immunoreactivity grade 1 is more closely associated, with L/T than is IgG; the difference between them, however, is not statistically significant.

Analysis of the average differences in I/W ratios for grade 1 insudation of albumin, IgG, and fibrinogen shows no statistically significant differences between them. These blood-derived components seem to be inexorably linked together in an initial increased endothelial permeability stage of arterial lesion formation. It should be noted that IgG at grade 1 is the insudation pattern.

It is proposed that the subsequent lipoprotein accumulation in the intima is an expression of a more severe alteration in endothelial permeability allowing the insudation of these extremely large globulins.[47] The arterial intima has a molecular sieve property, with small molecules passing faster than large ones.[48] Apo-B is a very large molecule that can combine with other molecules, such as fibrin[49] and C-reactive protein.[50–51] Even larger complexes can be formed by binding to elastic laminae[52] and glycosaminoglycans.[53] Disturbances in the sieve properties of the intima by the formation of such complexes would delay the passage of low density lipoproteins through the artery wall, with consequent modification of some of them.[49] The modified low density lipoproteins, in turn, would produce an imbalance between inflow and outflow of cholesterol, and thus could contribute to atheroma forma-

tion.[54] It is assumed, especially from experimentally induced atheroma studies, that lipid accumulation is the initiator of atherosclerosis.[1] However, in the present study of human coronary arteries, lipid deposits occur only after the changes in albumin, immunoglobulin, and fibrinogen insudation have happened. These results indicate that quite significant alterations to the coronary endothelium and intima have already occurred before lipid insudation becomes a factor in atherogenesis.

Artery lumen narrowing, expressed by the L/T ratio, is an important index of coronary heart disease, since the narrowed lumen is directly responsible for the clinical symptoms.[55] IgG immunoreactivity grade 1 is significantly different from grade 0 in I/W ratio, but shows no significant difference in L/T (Table 1). This suggests that immunoreactions may play a more important role in causing coronary intimal thickening than artery stenosis at the early stage of atherogenesis. Fibrinogen immunoreactivity grade 1 is not significantly different from that of grade 0 in terms of both I/W and L/T ratios (Table 1).

The correlation coefficients for intimal immunoreactivity grades and I/W ratios (Figure 11) show the relative significances of these substances in the development of atherosclerotic plaques. Statistically, they are all significantly positively correlated with intimal thickness. Albumin and apo-B have higher correlation coefficients than the others. Their presence would appear to be essential for increased intimal thickness. Altered albumin insudation is the earliest sign of arterial lesions developing. The presence of albumin, in turn, causes edema and swelling of the plaque, and a good deal of the plaque thickness can be attributed to such changes.[5]

The correlation coefficients of the immunoreactivity grades versus L/T are very similar to that for I/W (Figure 11), with albumin having the strongest and apo-B the next strongest correlation coefficients. Apo-B is more closely correlated with intimal thickness than it is with lumen stenosis.

It would appear, therefore, that atherosclerosis starts as a disease with features of both an inflammatory and an immune reaction, expressed by abnormal albumin insudation, immunoglobulin binding, and fibrinogen insudation. After these features, which are interpreted as showing a progressive increase in coronary endothelial permeability, are established, there is evidence of low density lipoprotein (apo-B) entry and accumulation in the intima. Regression coefficients indicate that albumin is the most prominent insudative factor involved in wall thickening and lumen narrowing, presumably through causing local edema.

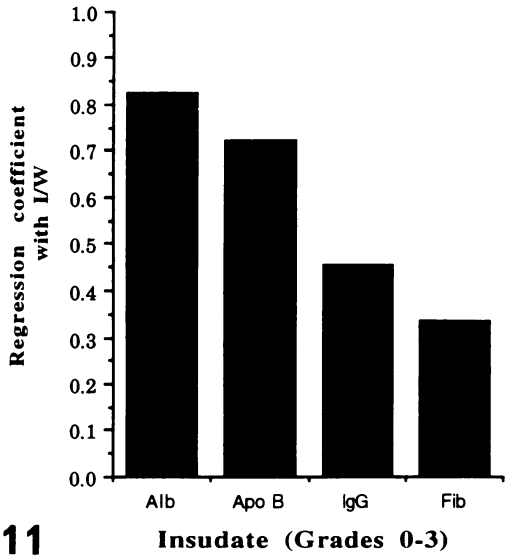

Figure 11. *Correlation coefficients (r) of intimal components immunoreactivity grades with I/W ratio.*

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