SUMMARY Apolipoproteins from human plasma high density (HDL), low density (LDL) and very low density lipoproteins (VLDL) were visualized in human arteries employing immunofluorescence techniques. Comparison between the localization patterns in extracranial and intracranial arteries and those in coronary arteries and the aorta was made. ApoA-I from HDL, apoB from LDL, and apoC-III from VLDL, as well as neutral lipid, were all localized to connective tissue and extracellular lipid pools in atherosclerotic lesions, and only to areas of intimal thickening in grossly "uninvolved" arteries.

SOME STUDIES in humans have implicated elevated levels of plasma low density (LDL) and very low density (VLDL) lipoproteins as risk factors for atherosclerosis in coronary as well as extracranial and intracranial artery beds. Dietary-controlled studies in experimental animals have corroborated this correlation. We studied localization patterns of LDL and of apoproteins from other plasma lipoproteins in human arteries. ApoA-I from HDL, apoB from LDL, and apoC-III from VLDL were localized in a number of different arterial beds in both uninvolvded and atherosclerotic regions and constituted indicators of lipid-protein accumulation. These results showed that these three apoproteins were primarily localized together with neutral lipid, and specifically to lesion connective tissue and atheromas. Similar results were obtained in a study of carotid artery bifurcations which were excised because of atheromatous involvement. In the present report we have extended previous studies for the purpose of ascertaining whether differences in prevalence of atherosclerosis in various vascular beds could be correlated with differences in lipoprotein accumulation.

Methods

The procedures for isolating apoA-I, apoB, and apoC-III from HDL, LDL, and VLDL, respectively, as well as the immunization procedures for raising specific antibodies were described previously. Globulin fractions of antibodies were first conjugated to fluorescein isothiocyanate (FITC) and then further purified by affinity chromatography on a column of Sepharose to which the appropriate antigen had been covalently coupled. The column thus functioned as a solid phase immunoadsorbent.

Segments from the following arteries were obtained at autopsy from 35 individuals ranging in age from 18 to 78 years: right and left middle cerebral arteries, the basilar artery, origin of the right and left internal carotid arteries; the right and left anterior descending, and left circumflex coronary arteries; and the ascending thoracic and abdominal aortas. These arteries were grouped into the following three categories: extracranial and intracranial arteries, coronary arteries, and the aorta.

The degree of superposition of localizations was similar in each vascular bed, and within the error resulting from the structural changes due to the focal nature of the atherosclerotic process. These results suggest a broad specificity in localization of apolipoproteins in most arterial lesions, and suggest that no differences in apolipoprotein accumulation exist between extracranial and intracranial arteries, coronary arteries, or the aorta. Variations in prevalence for atherosclerosis in each arterial bed must be accounted for on other bases.

The immunohistochemical procedures including control and test incubations have been described in detail previously. Serial sections were used due to the focal nature of atheromatous lesions.

Arteries were subdivided into the following groups: plaque, fatty streak, and uninvolved arteries. In our definition, plaques were focal arterial lesions demonstrating at least one major necrotic area; fatty streaks contained lipid accumulations but no necrosis; uninvolved arteries were lipid-free regions both with and without intimal thickening. Frequency in appearance within each group was recorded for the following: each individual apoprotein, superposition of localization of any two apoproteins, and superposition of localization of all three apoproteins.

Results

As was documented previously, apoA-I, apoB, and apoC-III and neutral lipid were found in the same regions of atherosclerotic arteries. These consisted of lipid cores or atheromas of plaques; in basilar (fig. 1a) or coronary arteries (figs. 1b and c) and along elastic fibers (fig. 1d).

In extracranial and intracranial arteries, 55% of plaques and 58% of fatty streaks demonstrated superposition of all three apoproteins and lipids (table 1). Eighty-nine percent of plaques and 83% of fatty streaks showed at least three out of the four factors together. In contrast, 76% of the uninvolved arteries from this arterial bed did not show presence of any of the four factors, while 96% showed no greater than one.

In coronary arteries, 65% of plaques and 67% of fatty streaks demonstrated the superposition of all four factors (table 1). Ninety percent of plaques and 84% of fatty streaks showed at least three out of the four factors together. Some apoproteins were found in the ten uninvolved arteries studied, always in areas of intimal thickening.

In the aorta, 54% of plaques and 44% of fatty streaks showed superposition of all four factors (table 1). Eighty-three percent of plaques and 88% of fatty streaks demonstrated at least three out of the four factors together.
As in the coronary arteries, some apoproteins were found in the four segments of uninvolved aortas, but always in areas of diffuse intimal thickening.

In extracranial and intracranial arteries—the frequency in the presence of apoproteins in plaques followed the progression: apoB > apoC-III > apoA-I (table 2). In fatty streaks the progression was: apoA-I > apoC-III > apoB. ApoA-I and apoB were found in four uninvolved arteries. Generally, the frequency in superposition of localization of any two apoproteins was the same in both plaques and fatty streaks. The superposition of localization of all three apoproteins likewise was almost identical in plaques and fatty streaks.

In coronary arteries the frequency in appearance of each apoprotein individually was almost identical, while in fatty streaks it frequently followed the progression: apoC-III > apoA-I > apoB. Seven out of the ten uninvolved coronary arteries showed apoB, always in diffuse intimal thickening. The frequency in appearance of any two apoproteins together was around 80% for both coronary plaques and fatty streaks, while the frequency of finding all three apoproteins together was 75% for both lesion types. Four out of ten uninvolved arteries also showed the superimposed localization of all three apoproteins, again in areas of diffuse intimal thickening.

In the aortic plaque the frequency in appearance of apoproteins was: apoB > apoC-III > apoA-I (table 2). In aortic fatty streaks it was: apoA-I > apoB = apoC-III. Two out of the four uninvolved aortas studied showed the presence of each apoprotein. The frequency in superposition of localization of any two apoproteins in aortic plaques followed the progression: apoC-III + apoA-I > apoB + apoC-III > apoB + apoA-I. Fifty-four percent of the aortic plaques and only 30% of aortic fatty streaks showed superimposed localization of all three apoproteins.

**Discussion**

This study represents an extension of a study reported earlier describing the frequency with which localization patterns of apoA-I from HDL, apoB from LDL, apoC-III from VLDL, and neutral lipid were superimposed.9

Only the atherosclerotic lesions consistently demonstrated
localization of all three apoproteins studied. Plaque connective
tissue and atheromas were the primary regions of
apoprotein accumulation. These results are consistent with
earlier studies on apoB and apoC-III localization from this
labatory* and others, and suggest that there exists an
affinity between the apoproteins and these lesion com-
ponents. It cannot be ruled out that neutral lipid deposited in
necrotic zones and along connective tissue fibers is the major
material interacting with apoproteins. Conversely, the lipid
moiety of the lipoprotein rather than the apoprotein may be
reacting with plaque components.

The major aim of this study was to ascertain whether any
differences could be found between the localization patterns of
apoproteins in extracranial and intracranial arteries and those in
corony arteries and the aorta of humans. Both the
frequency of maximum superposition of apoprotein and lipid
localizations and the frequency of finding all three
apoproteins together in atherosclerotic lesions were higher in
the coronary artery bed than in either the extracranial and
intracranial arterial bed or the aortic bed. It is conceivable
that this result may represent altered affinities for
apoproteins by different vascular beds. It appears more
likely, however, that both of these differences, as well as the
department within lesions from complete superposition of
localization patterns, result from the focal structural
changes in the artery due to the atherosclerotic process. It
seems quite likely that these focal changes are more abrupt
in smaller arteries such as intracranial arteries than in the
somewhat larger coronary arteries.

A large fraction of the arteries studied that were grossly
classified as uninvolved showed microscopically the presence
of lipid in the arterial intima, and were therefore put into the
fatty streak category, according to the definition employed in
this study. This resulted in an inordinately low number of
specimens in the uninvolved artery group, particularly for
coronary arteries and the aorta, making statistical evalua-
tion difficult. The results on uninvolved arteries, however, do
show that apoprotein accumulation occurs only in areas of
intimal thickening. Although extensiveness of positive
fluorescence for the three apoproteins was not as great in un-
involved arteries as in lesions, we must await quantitative
studies on such arteries to ascertain differences in apoprotein
accumulation.

Our results suggest that, aside from geometrical
differences in atherosclerotic lesions, there are no major
qualitative differences with respect to apoprotein accumu-
lation between the three vascular beds studied. The differences
in prevalence of human atherosclerosis between the extra-
cranial and intracranial arterial beds, and the coronary

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**Table 1** Frequency of Maximum Superposition of Apoproteins and Lipid in Human Arteries

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Total no. of specimens</th>
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<tr>
<td>Plaque</td>
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<tr>
<td>%</td>
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<tr>
<td>%</td>
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*Each arterial specimen was classified from 0 to 4 according to the
maximum number of apoproteins (apoA-I, apoB, and apoC-III) and neutral
lipid that had superimposed localizations in any major region of the artery.

**Table 2** Frequency of Apolipoprotein Localization and Superpositions in Human Arteries

<table>
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<tr>
<th>Specimen type</th>
<th>Total no. of specimens</th>
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<td>75</td>
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Fibromuscular Dysplasia and Multiple Dissecting Aneurysms of Intracranial Arteries

A Further Cause of Moyamoya Syndrome

P. Pilz, M.D.,* and H. J. Hartjes, M.D.†

SUMMARY A 16-year-old boy, who had sudden left-sided hemiplegia, died two weeks following onset of symptoms. A right carotid angiogram showed stenosis at the termination of the internal carotid artery. The middle cerebral artery had a beaded appearance and some of its branches were occluded. A basal “moyamoya” syndrome and transdural anastomoses were present. At autopsy, multiple intracranial dissecting aneurysms were found. Arteries of the body displayed fibromuscular dysplasia (FMD). The relevance of dysplastic changes of intracranial arteries and the relationship to moyamoya syndrome are discussed.

MANY REPORTS have been published in recent years on fibromuscular dysplasia (FMD) and moyamoya syndrome and they have been reviewed in the *Journal de Neuroradiologie*, volume 1, numbers 1 and 2, 1974. We present a case which shows features of both these conditions and, in addition, dissecting aneurysms of the intracranial arteries.

Case Report

RM, a 16-year-old boy, had had repeated attacks of middle ear infection and frontal sinusitis in recent years. The family history was unremarkable. On March 10, 1974, after throwing a snowball he suddenly had a severe headache and lost consciousness. After admission to the hospital he regained consciousness but was unable to move the left side of his body and he still had a headache. On physical examination he was alert and correctly orientated but a little euphoric. He laid on his left side and had no neck stiffness.

arterial and aortic beds, do not appear related to accumulation of apoproteins or their respective lipoproteins.

Acknowledgment

The author is indebted to the members of the Pathology Service, the Methodist Hospital for assistance in procuring arterial specimens, to Dr. Richard L. Jackson for providing purified apoproteins and their respective antibodies, and to Carol L. Heideman for providing excellent technical assistance.

References

Apolipoprotein localization in human cranial arteries, coronary arteries, and the aorta.

H F Hoff

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