Differing Patterns of Cholesterol Accumulation and \(^{3}\text{H}\)-Cholesterol Influx in Areas of the Cholesterol-fed Pig Aorta Identified by Evans Blue Dye\(^{1}\)

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In this study we have examined the possible focal and regional nature of aortic cholesterol accumulation in pigs receiving a cholesterol-containing diet for 6 to 16 weeks. Using uptake of the protein-binding azo dye Evans Blue as a marker for focal areas of increased aortic endothelial permeability \textit{in vivo}, we have found a significantly greater intimal accumulation of cholesterol in areas of dye uptake (blue areas) than in contiguous areas of the thoracic aorta showing no dye uptake (white areas). This focal difference in cholesterol accumulation was confined to the intima alone, and was not observed in either intima-media, or medial preparations. No measurable cholesterol accumulation was observed in the intima of blue relative to white areas in the absence of cholesterol feeding.

\(^{3}\text{H}\)-cholesterol was administered 72 hr before death to four pigs receiving a cholesterol diet for 6 weeks. Unesterified cholesterol radioactivity was found to be 60-70\% of the total activity in intimal or medial tissue. For each site studied (thoracic blue, thoracic white, and abdominal white), intimal cholesterol radioactivity was significantly greater than in the underlying media. Unesterified and esterified cholesterol radioactivity in intimal blue areas was significantly greater than the respective activities in the intima from thoracic white or abdominal white areas.

Cholesterol accumulation in the intima, but not in the intima-media alone, was found to exhibit a regional difference as well, with significantly greater accumulation in the thoracic than abdominal aortic segments. This regional pattern was also reflected in the \(^{3}\text{H}\) unesterified and esterified cholesterol activity in the intimal preparations from the thoracic and abdominal aortic segments.

This study has shown that aortic cholesterol accumulation is not homogeneous. The regional and focal difference in cholesterol accumulation observed may reflect local hemodynamic effects. The implications of these findings in terms of endothelial permeability to lipoproteins, and the pathogenesis of atherosclerosis are discussed.

INTRODUCTION

Atherosclerotic lesions in man and experimental animals are characteristically focal in distribution, (Mitchell and Schwartz, 1965) suggesting that local factors play a role in their pathogenesis. Previous studies have demonstrated that the aortic uptake of the protein-binding azo dye, Evans Blue, also

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FOCAL AORTIC CHOLESTEROL ACCUMULATION

exhibits a focal distribution (Friedman and Byers, 1963; McGill et al., 1957; Packham et al., 1967; Somer and Schwartz, 1971, 1972a), and that these areas of dye accumulation (blue areas) are areas of the increased in vivo permeability to $^{131}$I albumin (Packham et al., 1967; Bell et al., 1974a) and $^{131}$I fibrinogen (Bell et al., 1974b) relative to adjacent areas of the aorta showing no dye uptake (white areas).

Because of the similarity of the focal patterns of Evans Blue uptake in vivo to the focal distribution of atherosclerotic lesions in some species, (Friedman and Byers, 1963; McGill et al., 1957) a comparison of lipid metabolism between areas of high and low permeability is of particular interest. Studies by Somer et al., (1974) indicate that blue and white areas from the normal young pig aorta differ in their capacity to synthesize lipid from $^{14}$C acetate in vitro. Studies using $^{14}$C-labeled oleic acid and $^{32}$P-labeled phosphate in aortas from cholesterol-fed pigs indicated that cholesterol ester and phospholipid synthesis were both enhanced in blue areas compared with white in cholesterol-fed pigs although not in normal-fed pigs (Day et al., 1974). Somer and Schwartz (1971, 1972a) observed a greater uptake of intravenously injected $^{3}$H cholesterol in blue than in white areas of the pig aorta although no measurable difference in endogenous cholesterol content was observed between these areas of differing permeability. In the present report, aortas from normal pigs fed a cholesterol-containing diet for either 6 or 16 weeks were studied with respect to differences in cholesterol accumulation, and entry rates of isotopic cholesterol in focal areas of high and low permeability identified by Evans Blue dye in vivo.

MATERIALS AND METHODS

Yorkshire pigs were used for the studies. All pigs were weaned at 6 weeks of age and then maintained on a pelleted diet (Purina Hog Chow, Ralston Purina Co., Canada). The normal pigs used in the study were 8–10 weeks old and weighed 14–16 kg; cholesterol feeding was begun at this time and continued for either 6 or 16 weeks with body weights reaching 40–60 kg while on the diet. The cholesterol-fed pigs were fed a semi-synthetic high fat (28%), high cholesterol (2.8%) diet (Diet TD-70345, General Biochemicals, Chagrin Falls, OH) containing thiouracil prepared as described by Florentin et al., 1968). This diet resulted in plasma cholesterol levels of 774 ± 103 mg/100 ml (n = 6) after 6 weeks of feeding and an average of 1315 mg/100 ml (n = 2) after 16 weeks of feeding. The plasma cholesterol level in the normal pigs was 82 ± 5 mg/100 ml (n = 6).

Four animals fed cholesterol for 6 weeks were injected intravenously 72 hr prior to sacrifice with $^{3}$H cholesterol (generally labeled, Schwartz Bio Research, Orangeburg, NY) at a dosage of 0.125 mCi/kg body weight. The tracer was suspended in a mixture of ethanol, Tween 20, and 0.85% saline in the volume ratios 1:1:30 (Bell, 1973).

The pigs were injected via a jugular cannula with a 0.5% solution (w/v) of Evans Blue (Fisher Scientific Co., Fairlawn, NJ) in 0.85% saline at a dosage of 3 ml/kg body weight (Somer and Schwartz, 1971) and killed with an overdose of sodium pentobarbital 3 hr after injection of the dye. The intact aortas were removed from the aortic valve to the trifurcation, washed thoroughly with
AORTIC INTIMA-MEDIA CHOLESTEROL CONTENT IN DIFFERING SITES FROM PIGS FED EITHER A NORMAL DIET OR A CHOLESTEROL-CONTAINING DIET FOR 6 OR 16 WEEKS

<table>
<thead>
<tr>
<th>Area studied</th>
<th>Cholesterol content (µg/mg DNA)</th>
<th>Normal diet</th>
<th>Cholesterol diet</th>
<th>Cholesterol diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(6 wk)</td>
<td></td>
<td>(10 wk)</td>
</tr>
<tr>
<td>Thoracic blue</td>
<td>316 ± 26 (6)</td>
<td>924 ± 132 (6)</td>
<td>1556 (2)</td>
<td></td>
</tr>
<tr>
<td>Thoracic white</td>
<td>312 ± 33 (5)</td>
<td>747 ± 83 (6)</td>
<td>1414 (2)</td>
<td></td>
</tr>
<tr>
<td>Abdominal white</td>
<td>326 ± 58 (5)</td>
<td>544 ± 20 (5)</td>
<td>790 (2)</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± the standard errors of the mean. Number of animals is shown in parenthesis.

** Significantly different (P < 0.05) from thoracic blue or thoracic white values.

Tyrode’s solution, pH 7.4, and then stripped of periaventitial debris, after which they were opened longitudinally in the midline ventrally.

In the thoracic aorta, areas of Evans Blue accumulation (blue areas) and contiguous areas of no-dye accumulation (white areas) were excised and stripped of adventitia. Half of each tissue sample was taken for analysis of intima-media while the other half was further separated into intimal and medial layers at the cleavage plane near the internal elastic lamina for separate analysis of intima and media. Similar tissue samples were obtained from the abdominal aortic segment but these did not include any blued tissue since Evans Blue dye uptake is observed primarily in the thoracic aorta in the pig; in the abdominal aorta, bluing occurs only as small foci in close relation to the ostia of branches, (Packham et al., 1967; Somer and Schwartz, 1971). The intima-media samples and intima and medial layers were analysed separately. Surface area of intimal samples measured from 2-3 cm². Tissue lipids were extracted by homogenization in chloroform:methanol (2:1, v/v) and the extracts washed as described by Folch et al. (1957). Lipid-free tissue residues were used for the determination of deoxyribonucleic acid (Dische, 1955, Schneider, 1945). Lipid fractionation by thin-layer chromatography, cholesterol measurement and radioactive assay were performed as previously described in detail by Bell et al. (1970). Analyses of variance were performed on the appropriate data from the “blue” and the two “white” areas. Where statistical significance was present between the groups, P values were determined using Student’s t test. Isotopic data were subjected to a log transformation to make the data more normally distributed and to equalize the variance within the three tissue sites.

RESULTS

Table I shows the cholesterol content (µg/mg DNA) of intima-media aortic tissue from thoracic blue, thoracic white and abdominal white areas from normal pigs and pigs fed a cholesterol-containing diet for 6 or 16 weeks. Cholesterol feeding resulted in a progressive accumulation of cholesterol in each of the three arterial sites examined. Although in normal arteries there was no significant difference in the cholesterol content of thoracic blue, thoracic white and abdominal white segments, the cholesterol accumulated in the arteries of the cholesterol-fed pigs was not uniformly distributed. After six weeks on the
FOCAL AORTIC CHOLESTEROL ACCUMULATION

cholesterol-containing diet, the cholesterol content of both thoracic blue and thoracic white areas was significantly ($P < 0.05$) greater than that of abdominal white areas. Within the thoracic aorta, there was a tendency for blue areas to accumulate more cholesterol than the white areas, but this difference was not significant (Table I).

Although aortic cholesterol content was almost doubled between 6 and 16 weeks on the cholesterol-containing diet, the pattern of cholesterol accumulation after 16 weeks of cholesterol-feeding paralleled that observed at 6 weeks; i.e., the cholesterol content of blue and white thoracic segments was similar, and approximately twice that of abdominal white segments.

These data showed that cholesterol accumulation was greater in the thoracic aorta of the pig than in the abdominal aorta and, further, that within the thoracic aorta, no significant difference was observed between areas of high (blue) and low (white) permeability. However, when the cholesterol content of the intima and media from each of the three regions was analyzed separately (Table II), striking differences in the cholesterol content of the intima from areas of high and low permeability emerged. In the pigs fed the cholesterol-containing diet for 6 weeks, the cholesterol content of thoracic blue intima was approximately twice that of the thoracic white intima, a difference that is statistically significant ($P < 0.001$).

A similar two-fold difference in the cholesterol content of thoracic blue intima relative to thoracic white intima was also observed in the animals on the cholesterol-containing diet for 16 weeks (Table II).

Additionally, the cholesterol content of thoracic white intima was similar to that of abdominal white intima. As found in the intima-media tissues (Table I), no difference in cholesterol content was observed in the three sites

<table>
<thead>
<tr>
<th>TABLE II</th>
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<tbody>
<tr>
<td>AORTIC INTIMAL AND MEDIA CHOLESTEROL CONTENT IN DIFFERENT SITES FROM PIGS ON A NORMAL DIET OR A CHOLESTEROL-CONTAINING DIET FOR 6 OR 16 WEEKS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Areas and tissues studied</th>
<th>Cholesterol content ($\mu$g/mg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal diet</td>
</tr>
<tr>
<td>Intima</td>
<td></td>
</tr>
<tr>
<td>Thoracic blue</td>
<td>272 ± 29$^a$</td>
</tr>
<tr>
<td>Thoracic white</td>
<td>232 ± 30</td>
</tr>
<tr>
<td>Abdominal white</td>
<td>299 ± 41</td>
</tr>
<tr>
<td>Media</td>
<td></td>
</tr>
<tr>
<td>Thoracic blue</td>
<td>337 ± 23</td>
</tr>
<tr>
<td>Thoracic white</td>
<td>344 ± 37</td>
</tr>
<tr>
<td>Abdominal white</td>
<td>347 ± 48</td>
</tr>
</tbody>
</table>

$^a$ Mean ± the standard error of the mean.
$^b$ Mean of six animals.
$^c$ Mean of five animals—one lost in processing.
$^d$ Mean of two animals.
$^e$ Significantly different ($P < 0.001$) from thoracic or abdominal white values.
derived from the aortas of normal-fed pigs (Table II). These differences in intimal cholesterol content between thoracic blue and thoracic white areas of cholesterol-fed pigs were not reflected in the cholesterol content of media (Table II). Although medial cholesterol content increased progressively with the duration of cholesterol-feeding, as was observed in the intima and the intima-media (Table I), the medial cholesterol content of the three sites showed relatively slight variations (Table II).

Table III shows the content of isotopic cholesterol (cpm/mg DNA) in both intima and media of thoracic blue, thoracic white and abdominal white segments of pigs fed the cholesterol-containing diet for 6 weeks. The intima and media of all pigs contained both radioactive unesterified and esterified cholesterol. Regardless of the segment, unesterified cholesterol radioactivity was 60–70% of the total activity in the intimal or medial tissue. For each aortic site analysed, intimal cholesterol radioactivity was always significantly greater ($P < 0.001$) than in the underlying media. In comparing the radioactivity of the intima from each of the three sites, it was observed that both unesterified and esterified cholesterol radioactivity of intimal blue areas were significantly greater ($P < 0.001$) than that in thoracic white intima or abdominal white intima, which, in themselves, are not statistically different. No significant difference in either radioactive unesterified or esterified cholesterol was observed in comparing medial tissue derived from each of the three sites examined.

Consistent differences between intima and media were also apparent when total cholesterol (unesterified plus esterified) specific activity was examined (Table IV); intimal cholesterol specific activity was significantly ($P < 0.001$) greater than that of underlying media in each of the three sites. In addition, the total cholesterol specific activity of thoracic blue intima was significantly

### TABLE III

<table>
<thead>
<tr>
<th></th>
<th>Intima</th>
<th>Media</th>
<th>Overall</th>
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<tbody>
<tr>
<td></td>
<td>Esterified cholesterol (cpm/mg DNA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thoracic blue</td>
<td>3.14$^{b,c}$</td>
<td>0.47</td>
<td>1.22</td>
</tr>
<tr>
<td>Thoracic white</td>
<td>0.78$^{b}$</td>
<td>0.24</td>
<td>0.43</td>
</tr>
<tr>
<td>Abdominal white</td>
<td>0.37$^{b}$</td>
<td>0.17</td>
<td>0.25</td>
</tr>
<tr>
<td>Overall</td>
<td>0.96</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unesterified cholesterol (cpm/mg DNA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thoracic blue</td>
<td>7.94$^{b,c}$</td>
<td>1.22</td>
<td>2.94</td>
</tr>
<tr>
<td>Thoracic white</td>
<td>2.44$^{b}$</td>
<td>0.76</td>
<td>1.36</td>
</tr>
<tr>
<td>Abdominal white</td>
<td>1.80$^{b}$</td>
<td>0.68</td>
<td>1.12</td>
</tr>
<tr>
<td>Overall</td>
<td>3.17</td>
<td>0.86</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Values are the geometric means of the radioactivity from the aortas of four pigs receiving a cholesterol-containing diet for 6 weeks and injected with $[^3H]$-cholesterol (0.125 mCi/kg) 72 hr prior to death.

$^b$ Significantly different ($P < 0.001$) from corresponding values for media.

$^c$ Significantly different ($P < 0.001$) from thoracic white or abdominal white values.
FOCAL AORTIC CHOLESTEROL ACCUMULATION

TABLE IV
AORTIC INTIMAL AND MEDIAL TOTAL [3H]-CHOLESTEROL SPECIFIC ACTIVITY IN DIFFERING SITES FROM CHOLESTEROL-FED PIGS

<table>
<thead>
<tr>
<th></th>
<th>Intima cpm/μg cholesterol</th>
<th>Media cpm/μg cholesterol</th>
<th>Overall cpm/μg cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thoracic blue</td>
<td>5.20ab</td>
<td>2.52</td>
<td>3.62</td>
</tr>
<tr>
<td>Thoracic white</td>
<td>3.46bc</td>
<td>1.58</td>
<td>2.34</td>
</tr>
<tr>
<td>Abdominal white</td>
<td>3.23bc</td>
<td>1.53</td>
<td>2.22</td>
</tr>
<tr>
<td>Overall</td>
<td>3.87</td>
<td>1.83</td>
<td></td>
</tr>
</tbody>
</table>

* Values are the geometric means of the radioactivity from the aortas of four pigs receiving a cholesterol-containing diet for 6 weeks and injected with [3H]-cholesterol (0.125 mCi/kg) 72 hr prior to death.

b Significantly different (P < 0.001) from corresponding values for media.

c Significantly different (P < 0.05) from thoracic white or abdominal white values.

greater (P < 0.05) than that of either the thoracic white intima or abdominal white intima, which were similar. In contrast, there were no significant differences in total cholesterol specific activity of the medial tissues from the three sites.

DISCUSSION

In this study we have examined the topographical distribution of aortic cholesterol accumulation in both normal-fed pigs, and pigs fed a cholesterol-containing diet for either 6 or 16 weeks. It was found that aortic cholesterol accumulation in the cholesterol-fed pigs is not homogeneous but exhibits both focal (Table II) and regional (Table I, II) differences in distribution.

The focal nature of aortic intimal cholesterol accumulation is described in Table II. Accumulation was clearly greater in thoracic blue intima than in the intima derived from contiguous areas of the thoracic aorta showing no Evans Blue dye uptake. It is of interest that these focal differences in cholesterol accumulation were confined to the intima alone, and were not reflected in the media (Table II) nor in the intima-media (Table I). In contrast to this greater focal intimal cholesterol accumulation in areas of dye uptake in the cholesterol-fed pigs, no difference in cholesterol content between blue and white areas was found in pigs receiving a normal diet, with respect either to the intima-media, or to intima and media considered separately. The latter findings are consistent with the earlier observations of Somer and Schwartz (1971, 1972a) who found that the aortic cholesterol content of blue and white areas derived from the normo-cholesterolemic young pig is not measurably different, in spite of an enhanced uptake of 3H-unesterified cholesterol in blue areas.

The propensity of areas of Evans Blue dye uptake to accumulate cholesterol was also reflected in the isotopic studies in pigs receiving a cholesterol-containing diet for 6 weeks (Tables III and IV). Both 3H-unesterified and esterified cholesterol radioactivity (cpm/mg DNA) were significantly greater (P < 0.001) in thoracic blue than in thoracic white intima 72 hr after the intravenous injection of [3H]-cholesterol. Additionally, the radioactivity associated with both unesterified and esterified cholesterol was significantly greater (P <
0.001) in the intima than in the media, with no significant differences between media derived from thoracic blue, thoracic white, and abdominal white segments. The latter findings in the media appear to reflect the essentially similar cholesterol contents in the 3 sites from which the media was obtained (Table II). The enhanced uptake of isotopic cholesterol by the thoracic blue intima is further demonstrated by the significantly higher ($P < 0.05$) specific activity of total cholesterol (cpm/µg cholesterol) in the blue intima compared with either the thoracic white or abdominal white intima (Table IV). Although there was a tendency for total cholesterol specific activity to be greater in the thoracic blue media as well, it was not significantly different from that in either the thoracic or abdominal white media. This similarity in total cholesterol specific activity of medial tissue from the three sites probably reflects, again, the similarity in medial cholesterol content of the three sites (Table II).

These focal patterns of aortic intimal cholesterol accumulation described in Tables II, III and IV correspond with areas of in vivo Evans Blue dye uptake, areas which have previously been shown to be associated with enhanced permeability to radio-iodinated albumin (Packham et al., 1967; Bell et al., 1974a), and fibrinogen (Bell et al., 1974b). These areas of increased permeability in vivo are also associated with an increased endothelial cell turnover, as measured by $[^{3}H]$thymidine autoradiography (Caplan et al., 1973) that is considered to reflect hemodynamically-determined endothelial injury.

Apart from the focal blue and white differences outlined above, regional differences in cholesterol accumulation were also observed (Table I) with a greater accumulation of cholesterol in the thoracic than in the abdominal aortic segments. This regional pattern was also reflected in a significantly greater ($P < 0.001$) $[^{3}H]$ cholesterol activity associated with both unesterified and esterified cholesterol in thoracic blue intima (Table III). It is of interest that these regional differences in aortic cholesterol accumulation in pigs on a cholesterol-containing diet parallel the regional difference in permeability to $[^{125}I]$albumin (Bell et al., 1974a) and $[^{131}I]$fibrinogen (Bell et al., 1974b) Although our knowledge of the determinants of both focal and regional arterial differences in cholesterol accumulation and permeability is incomplete, experimental evidence in vivo and in vitro suggests that such differences in the arterial uptake of plasma constituents may relate to focal and regional differences in arterial wall strain or shear stress as a consequence of blood flow and pressure characteristics (Duncan et al., 1959; Duncan et al., 1965; Fry, 1968, 1969, Carew and Patel, 1973; Caro and Nerem, 1973; Caro, 1974). Whether the focal and regional differences relate solely to modification of or injury to the arterial endothelium, or in part reflect differences in the diffusion boundary layer as proposed by Caro et al. (1969, 1971) remains to be clarified.

The fact that no such focal or regional differences in cholesterol content was observed in normal arteries indicates that the factors suggested above may not themselves be sufficient to promote arterial wall accumulation of sterols in the absence of additional stress or stimulus such as the hypercholesterolemia ($> 700$ mg/100 ml) produced in the cholesterol-fed pigs of this study.

Some discussion on the possible role of endothelial permeability to lipoproteins as a determinant of the focal and regional differences in aortic cholesterol accumulation in the cholesterol-fed pig is relevant to this study. It is feasible
FOCAL AORTIC CHOLESTEROL ACCUMULATION

that the enhanced endothelial permeability in the normal pig aorta of blue
areas to macromolecules such as albumin and fibrinogen does not reflect a
general enhancement of permeability to all plasma proteins, including lipopro-
teins, but that the endothelium serves as a selective barrier to macromolecular
influx, discriminating on the basis of molecular size, molecular configuration
and the physical properties of the molecule itself. The lesser influx of $^{131}I$
albumin relative to $^{131}I$albumin (Bell et al., 1974a,b) is consistent with such
a discrimination. On the other hand, it is plausible that endothelial permeability
to lipoproteins is increased in blue areas of the normal artery, but lipoprotein
cholesterol does not accumulate because clearance mechanisms within the
arterial wall are adequate. In a previous study, (Somer and Schwartz, 1972a),
an increased uptake of tritiated cholesterol was described in blue areas of the
normal pig aorta; since a major proportion of the label was associated with
unesterified cholesterol as it is in the present study as well, it is likely that the
uptake observed was largely the result of a physicochemical exchange of
labeled unesterified cholesterol rather than lipoprotein entry. There is, however,
good evidence that normal arterial endothelium is permeable to circulating
lipoproteins, particularly low density lipoproteins (Smith et al., 1972, 1973).

In contrast to our observations in the normal aorta, there is no question
of an increased cholesterol accumulation in blue areas of the hypercholesterol-
emic pig. This could well reflect a net movement of lipoprotein from plasma
into the arterial wall resulting from several possible mechanisms. First, there
is a distinct possibility that endothelial permeability to plasma proteins is
increased in hypercholesterolemia. Our own studies have demonstrated an in-
creased endothelial permeability to $^{131}I$ albumin, in vitro, in aortas from pigs
fed cholesterol for 6 weeks ($^{131}I$ albumin entry was 45.5 and 28.3 µg/cm²/hr,
n = 2, in blue and white areas respectively in the cholesterol-fed pigs and
27.0 and 16.3 µg/cm²/hr (n = 4) in blue and white areas, respectively, from
normal pigs). These findings are consistent with the observations of Stefanovitch
and Gore (1971) who demonstrated an enhanced aortic permeability to albumin
in cholesterol-fed rabbits. In other words, hypercholesterolemia itself may
modify or injure the vascular endothelium, and thus impair its normal dis-
criminatory control over macromolecular influx. Additionally, the molecular
configuration and physical properties of low density lipoprotein may be suffi-
ciently modified in hypercholesterolemia to allow a more ready entry into
the arterial wall. While it is tempting to suggest that the focal and regional
differences in arterial cholesterol accumulation result from regional and focal
differences in endothelial permeability to lipoproteins, the importance of other
mechanisms cannot be excluded. For example, the possible role of arterial
endothelial lipoprotein lipase in generating cholesterol-rich remnants of lipopro-
teins on the arterial surface and their subsequent incorporation into the artery
is of interest (Zilversmit, 1973) particularly since there is some evidence that
arterial lipoprotein lipase shows focal differences in activity (Zemplenyi, 1968).
In addition, the possibility that the arterial wall can accumulate cholesterol
without macromolecular influx should be considered (Bell, 1974).

From the studies presented here, we are unable to say with any certainty
that the focal patterns of aortic intimal cholesterol accumulation represent sites
destined to develop atheroma. There is some evidence that in older pigs,
lesions do develop in the thoracic aorta at sites of in vivo Evans Blue accumulation (Murphy et al., 1962; Rowsell et al., 1965), and also in the abdominal aorta around the ostia of branches, which are characteristically areas of focal bluing. Detailed studies correlating the topography of areas of dye-uptake and sites of atheroma development should answer this question.

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REFERENCES


FOCAL AORTIC CHOLESTEROL ACCUMULATION


