Characterization of Cell Populations Isolated from Aortas of Rhesus Monkeys with Experimental Atherosclerosis

STANLEY FOWLER, PAUL A. BERBERIAN, HELEN SHIO, SIDNEY GOLDFISHER, AND HARVEY WOLINSKY

SUMMARY We used enzyme digestion to isolate cells from aortas of Rhesus monkeys (Macaca mulatta) fed on control or atherogenic diets for 19-21 months and subjected them to Metrizamide density gradient centrifugation. A majority of the cells from both control and experimental animals equilibrated in the density range of 1.08–1.12, but an additional population of cells (about 10% of the total) equilibrating at lower densities (ρ = 1.04–1.07) was present in preparations from diseased vessels. Except for a 10-fold increase in esterified cholesterol in cells from diseased aortas, the high density cells of both groups exhibited similar levels of marker enzymes of organelles (N-acetyl-β-glucosaminidase and β-galactosidase (lysosomes), neutral α-glucosidase (endoplasmic reticulum), 5'-nucleotidase (plasma membrane), and catalase (catalase-bearing particles)) and contained similar amounts of unesterified cholesterol. Electron microscopy revealed the high density cells as smooth muscle cells, with many of those from diseased vessels containing cytoplasmic lipid droplets and lipid-containing lysosomes. The latter were identified by acid phosphatase cytochemistry. The low density cells were enriched in both free and esterified cholesterol, acid hydrolases, and catalase. They appeared morphologically as foam cells heavily laden with lipid, which was present as cytoplasmic lipid droplets and as lipid-containing lysosomes. The cytoplasmic lipid droplets occupied 34–38% of the cytoplasmic volume of the foam cells. The proportion of total aortic cholesterol associated with cells was found to be 22.1 ± 7% (n = 3) in the control aortas and 17.0 ± 4% (n = 4) in the diseased aortas. Circ Res 46: 520–530, 1980

HYPERLIPIDEMIA is a major risk factor for cardiovascular disease (Kannel et al., 1971). Numerous experimental animal models exploring the association of hyperlipidemia and cardiovascular disease have been developed, including use of nonhuman primates fed on cholesterol-enriched diets (Gresham, 1976; Strong, 1976). Serum cholesterol levels comparable to those of western human populations can be attained in these animals and, in various branches of the primate vascular tree, lesions develop which resemble certain stages of human atherosclerosis. Among the primate models, experimental atherosclerosis in the Rhesus monkey has been the most studied, both in terms of the biochemical content (Portman and Alexander, 1966; Scott et al., 1967b; Manning and Clarkson, 1972; Wolinsky et al., 1975; Wagner and Salisbury, 1978) and the morphological appearance of the lesions (Scott et al., 1967a; Tucker et al., 1971; Stary, 1976). The interaction of serum lipids with vascular tissue which leads to the production of atherosclerosis remains to be elucidated fully. It is clear, however, that cells in the arterial wall play an important role in the tissue response to hyperlipoproteinemia. From rabbits fed a diet containing cholesterol, cells in varying stages of lipid accumulation can be isolated by enzymic digestion of atheromatous aortas and separated on the basis of their buoyant density in Metrizamide density gradients (Haley et al., 1977). These cells accumulate cholesterol both within lysosomes and as cytoplasmic lipid droplets, as revealed by ultrastructural cytochemistry and quantitative morphometry (Shio et al., 1974; Shio et al., 1978) and by subcellular fractionation of the cells (Peters and de Duve, 1974; Haley et al., 1977). Associated with this deposition are elevations in the levels of lysosomal hydrolases and also of catalase. Previous studies suggest that some of these phenomena also may occur in diseased arterial cells in monkeys and in humans (Goldfischer et al., 1975; Wolinsky et al., 1975; Coltof-Schiller et al., 1976; Berberian and Fowler, 1979).

In the study reported here, we apply the same cytological techniques used for the study of rabbit atheromata to the aortic lesions produced in Rhesus monkeys after cholesterol feeding for 19–21 months. Our findings are qualitatively similar to those obtained for rabbit atheromata, although quantitatively the data are quite different. We also demon-
strate that the major proportion of cholesterol that accumulates in aortas of monkeys maintained on the atherogenic regimen is located extracellularly.

Methods

Animals and Diet

Young adult female Rhesus monkeys (Macaca mulatta), weighing 4–12 kg at the start of the investigation, were fed a control or an atherogenic diet ad libitum for 19–21 months. The control diet consisted of Purina monkey chow and contained approximately 12% of the calories as fat, 70% as carbohydrate, and 18% as protein. The cholesterol content of this chow was less than 0.05% (Wolinsky et al., 1975). The atherogenic diet (Tucker et al., 1971) fed to the experimental group provided 40% of the calories as fat, 40% as carbohydrate, and 20% as protein; cholesterol was added to a final concentration of 0.37% (wt/wt). The serum cholesterol at the time we killed seven control monkeys averaged 123 mg/dl with a range of 98–144 mg/dl. Nine animals fed the atherogenic diet exhibited a wide range of serum cholesterol levels and, at the end of the feeding period, could be grouped into sets (three monkeys each) with moderate (201–373 mg/dl), high (422–476 mg/dl), or very high (686–874 mg/dl) serum concentrations, respectively. Serum triglyceride levels in the control monkey group averaged 20 mg/dl with a range of 9–44 mg/dl. Serum triglyceride levels in the experimental group were only slightly higher, averaging 29, 39, and 33 mg/dl for the moderate, high, and very high serum cholesterol subgroups, respectively. The animals were tranquillized with phencyclidine hydrochloride (2.5 mg/kg, im) (Sernylan, 20 mg/ml) and exsanguinated, and the entire length of the aorta from the aortic valve to the iliac bifurcation was removed for biochemical and morphological studies.

Isolation and Separation of Aortic Cells

Aortic cells were isolated by the collagenase and elastase tissue digestion procedure described by Haley et al. (1977). The excised aortas were immersed in glucose and amino acid-supplemented Hanks’ balanced salt solution (SH)* at 37°C and transported to the laboratory in a thermos bottle. The intima-media was carefully dissected away from the adventitia (Wolinsky and Daley, 1970) and then chopped into 0.5-mm squares with a McIlwain tissue chopper (Brinkmann Instruments). The chunks of intima-media were washed twice with collagenase solution (LCSH)* in which the calcium content had been reduced from the usual 2 mM to 0.2 mM. The ethanol contained in SVE serves to prevent the conversion of catalase to the inactive compound II.

* Special abbreviations used in this paper: SH medium, Hanks’ balanced salt solution (Hanks and Wallace, 1949) supplemented with glucose (5.6 mM), essential and nonessential amino acids (Jamieson, 1966), penicillin (100 IU/ml), streptomycin (100 µg/ml), and phenol red indicator; LCSH medium, SH medium with calcium content reduced to 0.2 mM; SVE medium, 250 mM sucrose containing 1 mM EDTA and 0.1% ethanol. The ethanol contained in SVE serves to prevent the conversion of catalase to the inactive compound II.

Subcellular Fractionation of Isolated Cells

Postnuclear supernatates of isolated aortic cells were fractionated by sucrose density gradient cen-
trifugation. Prior to homogenization, cells were washed twice in 250 mM sucrose containing 1 mM EDTA and 0.1% ethanol (SVE). Fractionations were performed in a Beaufay automatic zonal rotor using either the inner-layering (sedimentation) or the outer-layering (floation) technique as described by Peters and de Duve (1974).

Electron Microscopy

Specimens taken from atheromatous aortas were fixed in ice-cold 2.5% glutaraldehyde in 100 mM cacodylate buffer, pH 7.4, for 2 hours; postfixed 1.5 hours in 1% osmium tetroxide in 100 mM sodium cacodylate buffer, pH 7.4; and stained en bloc with uranyl acetate. After dehydration in graded alcohol and propylene oxide, the samples were embedded in Epon 812. Thick Epon sections (1 μm) were stained with methylene blue-azure II and photographed with a Zeiss universal photomicroscope using Kodak Panatomic-X film. Silver sections were cut on a Sorvall MT-2B ultramicrotome with a Dupont diamond knife, doubly stained with uranyl acetate and lead citrate, and viewed in a Philips EM-300 electron microscope operated at 80 kV.

Cytochemistry

Suspensions of isolated aortic cells were fixed (1:1 vol/vol) in 1.5% glutaraldehyde in 200 mM cacodylate buffer, pH 7.4, for 45 minutes at 0°C. After centrifugation in buffer to remove the fixative, the cells were suspended in the Gomori acid phosphatase medium, pH 5.0 (Barka and Anderson, 1962), and incubated at 37°C for 3 hours. The incubated cells were washed once in cacodylate buffer and then centrifuged with a Beckman 152 microfuge (Beckman Instruments). The tightly packed cell pellet was cut into small pieces, (postfixed in 1% osmium in 100 mM cacodylate buffer, pH 7.4,) and processed for electron microscopy as described above.

Quantitative Morphometry

The relative proportions of lipid present in foam cells within lysosomes or as cytoplasmic lipid droplets were determined by point-counting volumetry as described by Shio et al. (1978). On the basis of their distinctive morphology, foam cells were selected from a random sample of isolated cells not previously separated by density gradient centrifugation. Each preparation of isolated cells was fixed in ice-cold 2% glutaraldehyde in 100 mM cacodylate buffer, pH 7.4, for 30 minutes and then deposited onto Millipore filters (3-μm pore). The filtered cells were fixed in 1% osmium for 30 minutes and then processed for electron microscopy as above. From the Epon-embedded pellicles, ultrathin sections were prepared and the cells photographed. Only one ultrathin section per grid was selected, and the cells were photographed always in the upper right quadrant (in relation to the pellicle bottom). Point-counting volumetry was done with a multipurpose lattice test system consisting of 240 points overlaid on electron micrographs printed at a final magnification of 31,000X. After points lying over the nucleus and over extracellular spaces had been subtracted, the proportion of the remaining points found over lipid-filled lysosomes or over cytoplasmic lipid droplets was determined. Lipid-containing lysosomes were defined morphologically as membrane-bounded organelles filled with lipid and debris; lipid droplets were defined as nonmembrane-bound globules of lipid present in the cytoplasm.

Analytical Techniques

Glycosidases were assayed fluorometrically by the techniques of Peters et al. (1972) with the following incubation conditions employed: N-acetyl-β-glucosaminidase, 0.25 mM 4-methyl umbelliferyl-2-acetamido-2-deoxy-β-D-glucopyranoside, 50 mM sodium citrate, pH 4.8, and 0.1% Triton X-100; β-galactosidase, 0.25 mM 4-methyl umbelliferyl-β-D-galactoside, 50 mM sodium formate, pH 4.3, 5 mM MgCl₂, and 0.1% Triton X-100; neutral α-glucosidase, 1 mM 4-methyl umbelliferyl-α-D-glucopyranoside, 50 mM sodium phosphate buffer, pH 6.8, and 0.1% Triton X-100. Assays for cytochrome oxidase, 5'-nucleotidase, and catalase were performed as described by Peters et al. (1972), except that the incubation for catalase was done at 0°C for 60 minutes. Each enzyme assay was checked on aortic cell homogenates for linearity with time and enzyme concentration. For all hydrolases, 1 unit of activity corresponds to the hydrolysis of 1 μmol of substrate per minute at 37°C. Catalase activity is expressed in the units of Baudhuin et al. (1964).

Free and esterified cholesterol were measured quantitatively by gas-liquid chromatography as described by Haley et al. (1977). DNA was assayed by the fluorometric procedure of Kissane and Robins (1958) with calf thymus DNA used as standard.

Presentation of Results

Results of the density gradient experiments are presented in histogram form as a function of the gradient volume (de Duve, 1967). The ordinate is the relative concentration that the enzyme would have if it were homogeneously distributed throughout the gradient. The shape of the gradient is given separately and, to facilitate interpretation, the starting conditions are also depicted on the diagrams. Morphometric results are expressed as percentages of total cytoplasmic volume.

Statistical analyses of biochemical data, t-statistic evaluations (paired observations and two-sample test) and determinations of correlation coefficients, was carried out as described by Snedecor and Cochran (1967).

Results

The aortic lesions produced in the monkeys fed the atherogenic diet for 19–21 months were similar to those described by others (Scott et al., 1967a;
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IEL—

M

FIGURE 1 Light microscopic appearance of a typical lesion in the thoracic aorta of a monkey fed on an atherogenic diet for 19 months. The lesion consists of an intimal thickening (I) containing foam cells situated just beneath the endothelium and smooth muscle cells embedded in a fibrous matrix. Cells in the media (M) appear relatively unaffected. IEL = internal elastic lamina. Bar = 20 μm.

Armstrong and Warner, 1971; Tucker et al., 1971; Manning and Clarkson, 1972; Chakaravarti et al., 1976) and consisted mostly of yellow fatty streaks with some raised, flat, white-yellow plaques also present. Together, these two types of lesions covered 50-75% of the luminal surface of the aortas. Histologically, the lesions appeared as intimal thickenings containing numerous foam cells, usually concentrated just beneath the endothelium, and many easily identifiable smooth muscle cells (Fig. 1). The media, comprising the major mass of the tissue used in our studies, appeared largely unaffected. Flat, diffuse fibromuscular intimal lesions occasionally were observed in the abdominal portions of aortas from monkeys fed the control diet. Oil red O staining of frozen tissue sections for neutral lipid showed diffuse small droplets throughout the lesions, with focal collections of large globules also present; intra- vs. extracellular localization could not be clearly determined in the stained sections.

Biochemical Studies

Aortic Homogenates

The mean contents of DNA and cholesterol and the activities of various cellular enzymes measured in representative samples of homogenized aortas taken from four control and four experimental animals are presented in Table 1. The DNA contents per mg tissue wet weight of control and diseased aortic preparations were essentially the same, indicating a similar overall cellularity of the aortas. Although large variation in lipid content among individual aortas was found, the mean level of cholesterol in aortas from experimental monkeys was about 3 times greater than the mean of the controls, and the amount of esterified cholesterol was increased to about the same degree. The absolute cholesterol values that we obtained are the same or somewhat higher than those reported by Mohan and Chakravarti (1975), Scott et al. (1967b), and Wolinsky et al. (1975). A rough correlation, although not statistically significant, between individual serum cholesterol levels and aortic cholesterol content was found in our study. Measurement of five marker enzymes representing four subcellular organelles did not reveal significant differences in

<table>
<thead>
<tr>
<th>Table 1 Biochemical Analyses of Monkey Aortic Homogenates*</th>
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<tr>
<td>Control group†</td>
</tr>
<tr>
<td>(n = 4)</td>
</tr>
<tr>
<td>DNA (mg/g wet wt‡)</td>
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<tr>
<td>Cholesterol (mg/mg DNA)</td>
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<tr>
<td>Total</td>
</tr>
<tr>
<td>Free</td>
</tr>
<tr>
<td>Esterified</td>
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<tr>
<td>Enzymes (mU/mg DNA)</td>
</tr>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
</tr>
<tr>
<td>β-Galactosidase</td>
</tr>
<tr>
<td>Catalase</td>
</tr>
<tr>
<td>Neutral α-glucosidase</td>
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<tr>
<td>5'-Nucleotidase</td>
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* Each aorta, stripped free of adventitia, was chopped into 0.5-mm squares with a tissue chopper, and the cubes were mixed thoroughly. Representative samples of known weight then were taken and homogenized in SVE with a Dull glass homogenizer. Values obtained are expressed as means ± sd.
† Serum cholesterol values at the time that we killed the monkeys were 121 ± 20 mg/dl for the control group and 479 ± 280 mg/dl for the experimental group.
‡ Wet weights of the intima-media layers of the whole aortas were 1.55 ± 0.7 g for the control group and 2.30 ± 0.9 g for the experimental group.
their activities, based on DNA, between diseased and control aortic homogenates.

Isolated Cells

Cells isolated from monkey aortas were separated according to their density by Metrizamide gradient centrifugation. Representative experiments from the control and experimental animals are presented for comparison (Fig. 2). As revealed by the distribution of DNA, most cells equilibrated at a relatively high density; in 10 experiments (four control, six experimental), the modal equilibrium density was $1.10 \pm 0.01$. A small number of cells (less than 10% of the total) isolated from diseased aortas equilibrated at densities less than 1.07, whereas none from control aortas were found at this density. These cells are observed as a skewing of the left side of the distribution profile of DNA for the experimental cells (Fig. 2). They are rich in both free and esterified cholesterol as shown by the bimodal distributions of these lipids in the density gradient. The low density cells also contain high activities of two lysosomal enzymes, \( \text{N-acetyl-} \beta\text{-glucosaminidase} \) and \( \beta\text{-galactosidase} \), as well as of catalase. Table 2 summarizes the results from the 10 experiments and demonstrates a consistent enrichment of the low density cells with cholesterol, lysosomal enzymes, and catalase. Except for a 10-fold increase in esterified cholesterol, the high density cells exhibited enzyme and free cholesterol levels similar to those of control cells.

Based on cholesterol contents of the isolated cells and of the aorta from which they were isolated, the percentage of the total vessel cholesterol present within the cells could be calculated. The data presented in Table 3 indicate that approximately 20% of total cholesterol in the aortic wall is associated with cells in both control or experimental animals.

Subcellular Fractionation

Subcellular fractionation of cell homogenates in sucrose density gradients revealed no significant differences in the modal densities and distribution profiles of lysosomes, microsomes, mitochondria, plasma membrane, or particles bearing catalase of aortic cells derived from either control or experimental animals. This was true whether the organelles were separated by flotation (Fig. 3) or by sedimentation technique (Peters and de Duve, 1974); one control aorta and one experimental aorta.

![Figure 2 Isopycnic centrifugation in Metrizamide gradient of isolated aortic cells from single animals fed either on control or experimental diet. The graphs show distributions of constituents and enzymes as a function of gradient volume. Density gradient is shown by the “staircase.” Shaded area represents initial distribution of cells. Recoveries of constituents and enzymes in the gradient ranged from 75 to 116%.

<table>
<thead>
<tr>
<th>Control</th>
<th>Experimental</th>
</tr>
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<tbody>
<tr>
<td>DNA</td>
<td>Total Cholesterol</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
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</table>
| \( \text{N-acetyl-} \beta\text{-glucosaminidase} \) | \( \beta\text{-galactosidase} \) | Catalase | }

Percent Volume

Density

Relative Concentration

Percent Volume
TABLE 2 Biochemical Characteristics of Monkey Aortic Cell Populations Separated by Isopycnic Centrifugation *

<table>
<thead>
<tr>
<th></th>
<th>Control group (n = 4)</th>
<th>Experimental group (n = 6)</th>
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<tbody>
<tr>
<td></td>
<td>Control cells (p = 1.070-1.150)</td>
<td>High density cells (p = 1.070-1.150)</td>
</tr>
<tr>
<td>DNA</td>
<td>0.114 ± 0.06</td>
<td>0.137 ± 0.005</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>0.96 ± 0.2</td>
<td>1.01 ± 0.6</td>
</tr>
<tr>
<td>Esterified cholesterol</td>
<td>&lt;0.05</td>
<td>0.53 ± 0.6</td>
</tr>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>124 ± 30</td>
<td>179 ± 75</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>37.3 ± 24</td>
<td>27.8 ± 8</td>
</tr>
<tr>
<td>Catalase</td>
<td>0.295 ± 0.09</td>
<td>0.233 ± 0.05</td>
</tr>
<tr>
<td>5'-Nucleotidase</td>
<td>8,240 ± 2,400</td>
<td>8,890 ± 2,760</td>
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</table>

* Data are means ± SD. Recoveries of constituents in gradients averaged 87% (range 64-116) in the control group and 89% (range 63-137) in the experimental group.
† Specific content is expressed for DNA as mg/g aorta and for other constituents as mg or mU/mg DNA.
‡ Differences between low density and high density cells are statistically different at P < 0.005 calculated by paired t-test.

were studied by each technique. The flotation experiments in particular gave no indication of the presence of low density lysosomes similar to those in rabbit atheromata as described by Peters and de Duve (1974) and Haley et al. (1977). Unfortunately, too few low density cells were isolated to conduct the subcellular fractionation studies on this subpopulation of cells.

Morphological Studies

Cells isolated from diseased vessels appeared as a heterogeneous mixture of cells. After Oil red O staining, a few cells stained intensely red indicating heavy lipid deposition, whereas other cells showed moderate lipid accumulation. Many cells, however, revealed only a fine punctate staining of the cytoplasm.

By electron microscopy, isolated cells that had equilibrated at high density exhibited the typical features of smooth muscle cells, including abundant myofilaments and numerous pinocytic vesicles. Lipid inclusions in these cells were frequently observed (Fig. 4, a and b). After acid phosphatase staining, lysosomes in the cytoplasm were identified, and accumulation of lipids within them was evident (Fig. 4c).

Isolated cells that had equilibrated at low density were filled with lipid inclusions and appeared as typical foam cells (Fig. 5a). Acid phosphatase staining revealed the presence of two kinds of lipid inclusions, lipid-containing lysosomes and cytoplasmic lipid droplets (Fig. 5, b and c). Morphometric analyses of the foam cells showed over half of the cell cytoplasm to be occupied by lipid inclusions; cytoplasmic lipid droplets accounted for nearly two-thirds of the lipid volume (Table 4).

Discussion

We have described several cellular and tissue alterations found in the aortas of Rhesus monkeys.
Figure 4  Ultrastructure of isolated monkey cells collected from high density regions of a Metrizamide gradient. Examples of lipid inclusions found in cells from diseased vessels are seen in a and b. Lipid accumulation occurs in the form of lipid droplets (D) or membrane-bounded vacuoles (Ly). The latter are identified as lysosomes by cytochemical staining for acid phosphatase as shown in c. The lipid-containing lysosomes exhibit a variable morphology as illustrated in examples presented in insets. N = nucleus. a = 13,000x; b = 23,000x; c = 18,000x; insets: lower left = 44,000x; upper right = 34,000x.
Figure 5  Appearance of low density cells obtained from a diseased monkey aorta. a: These cells are filled with lipid inclusions and appear as typical foam cells. After cytochemical staining for acid phosphatase (b and c), lipid-filled lysosomes (Ly) can be identified and distinguished from nonreactive membrane-free cytoplasmic lipid droplets (D). N = nucleus; Ga = Golgi. a = 10,000x; b = 19,000x; c = 16,000x.

after they were fed on a cholesterol-rich diet for nearly 2 years. The dietary regimen resulted in serum cholesterol levels of 200–800 mg/dl, comparable to those measured in humans seen in clinical practice. The lesions induced in the monkey aortas were not greatly different from the fatty streaks and plaques observed in young adult humans at autopsy. Lipid overloading of the monkey aortic
cells was observed and was most dramatic in the low density foam cells which were few in number and present only in the diseased vessels. It was evident also in the more numerous high density cells identified as arterial smooth muscle cells. The lipid overloading takes its form in the appearance of cytoplasmic lipid droplets and lipid-filled lysosomes. This process is associated with the accumulation of free and esterified cholesterol and, at least in the low density cells, with elevated levels of lysosomal enzymes and catalase. Even in the absence of large experimental groups that can be subjected to detailed statistical evaluation, it is still possible to compare these results to studies of a similar kind on experimental diet-induced disease in rabbits and to spontaneous atherosclerosis in man.

Rabbits fed a cholesterol-enriched chow sustain elevations of serum cholesterol levels measured in thousands of milligrams per deciliter and develop severe diffuse atherosclerosis. Up to one-half of the cells isolated from the rabbit atheroma can be found to be massively enriched in lipid, catalase, and lysosomal enzymes (Haley et al., 1977; Fowler et al., 1979). When homogenates of these cells are subjected to isopycnic centrifugation on sucrose density gradients, a population of low density lipid-filled lysosomes is readily demonstrated (Peters and de Duve, 1974; Haley et al., 1977). Morphometric analysis of rabbit foam cells shows cellular lipid content to be about equally distributed between lipid-containing lysosomes and cytoplasmic lipid droplets (Shio et al., 1978); excess free cholesterol accumulates in the former, and much of the cellular esterified cholesterol is present in the latter (Shio et al., 1979).

In hyperlipemic Rhesus monkeys, a similar kind of lipid accumulation occurs in the arterial cells in association with serum lipid concentrations much lower than occur in the rabbit. Compared to the rabbit lesion, far fewer of the cells are heavily involved, and the accompanying increases in cellular lipid and enzyme levels are smaller. Also, cytoplasmic lipid droplets account for a substantially greater proportion of the lipid inclusion volume in the monkey low density cells than was observed previously in the comparable rabbit low density cell population (Shio et al., 1978). No low density lysosomes were detected by subcellular fractionation of the monkey cells, although such lysosomes simply may have been too few in number to be detected by our fractionation techniques. It should be kept in mind that a substantial proportion of the isolated cells that we obtained from diseased aortas probably were derived from the relatively unaffected medial portion of the vessels. The importance of the cellular abnormalities observed in the primate lesion, however, should not be diminished because of their subtlety relative to the rabbit lesions; rather, together, the two experimental models serve to put into perspective the human lesion, which no doubt will remain the most difficult to study in comparable detail.

In spontaneous atherosclerosis in man, biochemical analyses of diseased vessels have shown increased activities of lysosomal acid hydrolases (Miller and Kothari, 1969; Platt, 1970), and morphological studies of the vessels have shown the presence of lipid in lysosomes (Coltoff-Schiller et al., 1976; Fowler et al., 1978). A recent systematic study of human arterial lesions established that when homogenates of selected areas are compared with uninvolved regions, enrichment in free and esterified cholesterol, lysosomal enzymes, and catalase are found in the lesions; marker enzymes of other subcellular organelles and the relative content of DNA per mg tissue wet weight are not changed (Berberian and Fowler, 1979). Thus, the same pattern of marker enzyme activities is present in man as occurs in experimental atherosclerosis in rabbits and monkeys. However, the degree of biochemical alteration found in human lesions was much less than that of the experimental rabbit lesion, but greater than that recorded here for the less diseased monkey aortas.

In the present study, biochemical (Table 2) and morphological (Table 4) analyses both indicate that the most markedly altered cells, the foam cells, amount to 7 or 8% of the total isolated cell population. Given the many medial layers of uninvolved tissue included in the sampling, it is difficult to know what importance to assign this cell type, particularly since many of the high density cells of the diseased aortas also were considerably enriched in lipid, although not changed in buoyant density. The high density cells clearly are smooth muscle cells, whereas the origin of foam cells remains unclear; certain cellular features favor a macrophage origin and others a smooth muscle cell origin (Scott et al., 1967a; Stary, 1976; Fowler et al., 1979; Schaffner et al., 1979).

Finally, we also have investigated the question of

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Lipid-containing lysosomes</th>
<th>Cytoplasmic lipid droplets</th>
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<tbody>
<tr>
<td>1</td>
<td>7.8</td>
<td>19 ± 5</td>
</tr>
<tr>
<td></td>
<td>(28)</td>
<td>(28)</td>
</tr>
<tr>
<td>2</td>
<td>5.7</td>
<td>18 ± 6</td>
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<td>(20)</td>
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<tr>
<td>3</td>
<td>7.1</td>
<td>23 ± 6</td>
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<td></td>
<td>(18)</td>
<td>(18)</td>
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</table>

* Determined from cell counts made on thick Epon sections of embedded isolated cells.

† Values are expressed as means ± SD with number of cell profiles examined indicated in parentheses.

Morphometric Analyses of Foam Cells Isolated from Experimental Monkey Aortas
location of lipid in the arterial wall. The preponderance of cholesterol in both the normal and diseased vessels was found to be extracellular. It is not easy to identify possible sources of major artifact in our methodology, although selective loss of a lipid-rich population of cells during the cell isolation procedure is one possibility. If one accepts the validity of the results obtained on seven animals over a broad range of aortic lipid contents, what are the implications? It could be argued that most of the lipid now being measured was originally present intracellularly, having been released into the extracellular space through extensive cellular necrosis. Cell death is a significant component of the lesions seen in Rhesus monkeys (Scott et al., 1967a). Another possibility is that most of the vascular lipid is located in the extracellular matrix from the very onset of the lesion, as has been suggested by earlier studies of cholesterol ester fatty acid patterns in atherosclerotic vessels from patients (Smith, 1974). If extracellular lipid is indeed preponderant in vessels from the onset of disease, it would appear that strategies of intervention aimed at either slowing progression or promoting regression are likely to be very different from those involving manipulation of cellular functions. Therefore, it will be of great interest to determine whether the present results can be verified by similar quantitative studies of human disease and in experimental lesions at various stages of development in other animal models.

Acknowledgments

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References


Shio H, Haley NJ, Fowler S (1979) Characterization of lipid-laden aortic cells from cholesterol-fed rabbits. III. Intracellular...
localization of cholesterol and cholesteryl ester. Lab Invest 41: 160-167
Wagner WD, Salisbury BGJ (1978) Aortic total glycosaminoglycan and dermatan sulfate changes in atherosclerotic Rhesus monkeys. Lab Invest 39: 322-328

**Time Course of Changes in the Mechanical Properties of the Canine Right and Left Ventricles during Hypertrophy Caused by Pressure Overload**

**ISRAEL MIRSKY AND MICHAEL M. LAKS**

**SUMMARY** We developed a mathematical model of the right and left ventricles to determine whether there is a change in the mechanical properties of muscle during the hypertrophy process resulting from pulmonary arterial banding. Pressure-volume data were obtained from 10 normal dog hearts and 8 dog hearts in which the pulmonary artery was banded for periods of 2-40 weeks. These data were applied to the model, and the time course of wall stress and muscle stiffness was quantified for both ventricles. The results demonstrate that (1) myocardial stiffness is increased in pressure-overload hypertrophy (2) normal right and left ventricular muscle exhibits similar mechanical properties and (3) the relationships between wall stresses and the volume/mass ratios to the period of banding are biphasic. We concluded that (1) increase in muscle stiffness is due to several factors. In the early stages of hypertrophy, it may be predominantly due to fibrosis and, in the later stages, to substantial increases in muscle mass. (2) The progressive increase in muscle stiffness concomitant with the increase in muscle mass may be due to the presence of myocardial cellular projections and fibrosis. (3) The appropriate timing for surgical/medical intervention should take place before low volume: mass ratios and, hence, low wall stresses are attained. Circ Res 46: 530-542, 1980

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**THE ASSESSMENT** of the diastolic properties of the left ventricle continues to be a subject of great interest, although beset with controversy (Levine, 1972; Spotnitz and Sonnenblick, 1973; Covell and Ross, 1973; Mirsky, 1976; Glantz and Parmley, 1978). No doubt much of the controversy arises from the fact that the role of the right ventricle and the pericardium has been neglected. Since the right and left ventricles share a common wall—the sep-

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Wagner WD, Salisbury BGJ (1978) Aortic total glycosaminoglycan and dermatan sulfate changes in atherosclerotic Rhesus monkeys. Lab Invest 39: 322-328

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Recently, Mirsky and Laks (in press) developed a geometric model for the heart and examined the time course of stress during the development of
Characterization of cell populations isolated from aortas of rhesus monkeys with experimental atherosclerosis.

S Fowler, P A Berberian, H Shio, S Goldfischer and H Wolinsky

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