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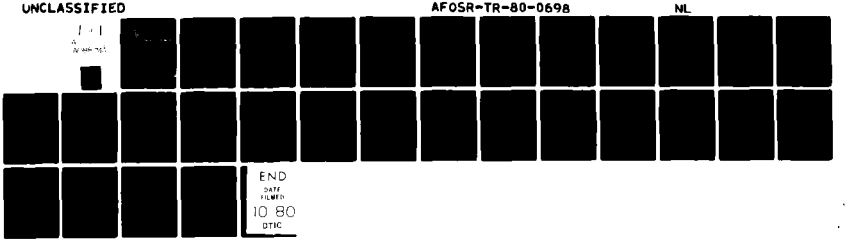

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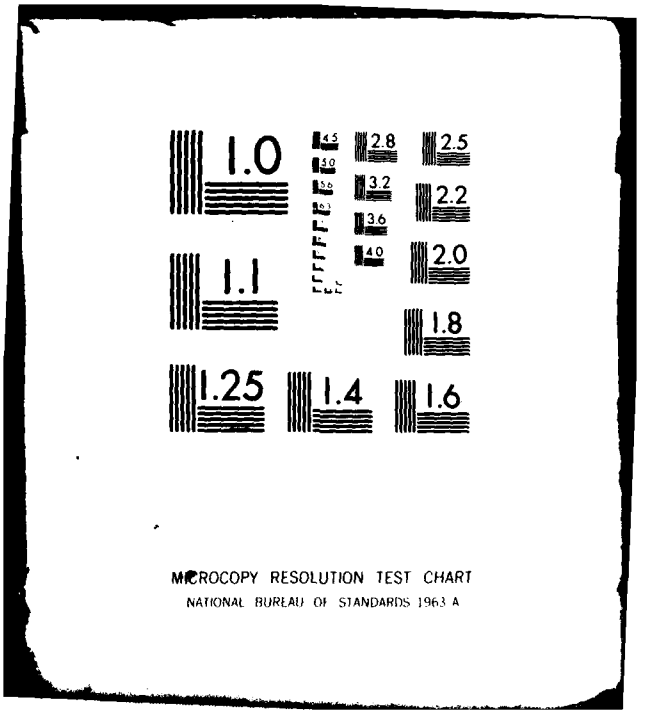
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CARDIOVASCULAR, RENAL AND RESPIRATORY EFFECTS OF HIGH INTENSITY, INTERMEDIATE DURATION, LOW FREQUENCY VIBRATION

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For the Period
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U.S. AIR FORCE
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A research program on the influence of high intensity, intermediate duration, low frequency wholebody vibration on the cardiovascular, renal and respiratory systems is described. During the period 1 October 1978 to 31 December 1979, the major emphasis was on the development of quantitative methods to be used in the study of wholebody vibration effects. The methods developed include a television image processing system for the quantification of the pattern of atherosclerosis and a vascular casting technique for the study of →

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systemic arterial endothelium. These techniques are not only suitable for use in the study of wholebody vibration effects, but have a larger range of applicability which includes cardiovascular physiology in general and the study of environmental factors on the cardiovascular system.

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The experiments reported herein were conducted in accordance with the principles in the Guide for the Care and Use of Laboratory Animals [DHEW Publication No. (NIH) 78-23].

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I. Introduction

As part of its recognized interest over the years in the limiting factors on pilot performance, the U.S. Air Force has of necessity been interested in physiological behavior as related to the conditions in which its personnel must operate. One such area has been the man/machine interface problem associated with placing flight personnel in a vibration environment such as in an airplane or helicopter.

In attempting to understand the nature of any hazard to which personnel are exposed when placed in such an environment, i.e. one involving low frequency, wholebody vibration, it is important to understand the long term subtle effects and not just the immediate readily observable ones. It is to these questions that the present research effort is addressed. This effort was originally supported under AFOSR Grant No. 73-2526 entitled "Cardiovascular, Renal and Respiratory Effects on High Intensity Intermediate Duration, Low Frequency Vibration." The present effort is an extension of those earlier efforts and has been supported by AFOSR Grant No. 77-3411. In this report, the results obtained to date on the present grant are outlined. It is believed that these results represent a significant start on a rather unique and important problem, i.e. the development of quantitative techniques for the study of the arterial wall.

II. QUANTIFICATION OF ARTERIAL LESION TOPOGRAPHY

The determination of the effect of various factors on the etiology of atherosclerosis requires quantitative methods to evaluate objectively both the extent and location of arterial lesions and the morphology of arterial endothelial cells. If such techniques were available, it would be possible to conduct sequential studies on the progression and regression of both spontaneous and experimental atherosclerosis. In addition, the effect of environmental factors and such different stimuli as hemodynamic forces, pharmacological agents, hormonal levels, etc. could be investigated. The purpose of this section is to present two quantitative techniques developed in our laboratory for the study of arterial lesions. The first of these is the polar coordinate technique used to study both ostial lesions (sudanophilic and Evans Blue Dye uptake) and ostial endothelial repair. The second is the television-computer scanning system used to study the topology of lesions over an entire arterial surface.

The predilection for atherosclerotic lesions to occur at branch points and the need for quantitation of these lesions led to the development of the polar coordinate technique of analysis of periorificial lesions by Cornhill and Roach (1, 2). This technique determines quantitatively the exact location and extent of lesions with respect to an arterial ostia. In brief, the orifice was centered on the polar coordinate grid such that 0° was proximal to the ostia (i.e. direction of blood flow), 90° was to the animals left, 180° was distal (downstream), 270° was to the animals right and 360°, being the same point as 0°, was proximal. The distance between the lip of the orifice and the periphery of the lesion was measured at 5° or 10° intervals and plotted on rectangular coordinates. The technique is automated using a digitizer and additional calculations of ostial and lesion areas are made. The differences between groups may easily be determined using the two way analysis of variance. In studies of experimental atherosclerosis in the hypercholesterolemic rabbit, sudanophilic lesions in the descending aorta are found initially in areas distal to the flow divider (2). By contrast, spontaneous atherosclerotic lesions, occurring at the coeliac ostia of the White Carneau Pigeon, are found initially in areas proximal to the flow divider (3). Figure 1 illustrates the mean of 5 birds in each of three groups (age 1, 4 and 6.5 yrs.) and shows that even with advanced lesions the coeliac lesions have almost no distal components. The different location of development of the experimental and spontaneous lesions raises important questions regarding the importance of hemodynamic forces - in this case wall shear stress - in atherogenesis. The experimental sudanophilic lesions in the hypercholesterolemic rabbit occur distal to the branches in areas expected to experience relatively high wall shear stress; whereas, the spontaneous lesions in the White Carneau Pigeon occur proximal to the bifurcation in an area expected to experience relatively low wall shear stress. The apparent dichotomy of these findings warrants important consideration not only

of the role of wall shear stress in atherogenesis, but in its possibly different role in the development of experimental as opposed to spontaneous lesions.

The polar coordinate technique has also been used to study the areas of increased albumin transport (Evans Blue Dye uptake) and endothelial regeneration subsequent to balloon catherization.

In a separate effort a television image processing system originally designed and built by J.M. Jagadeesh (Ph.D. Dissertation, The Ohio State University, 1974) has been adapted and applied to the particular problem of identifying sudanophilic lesions. Aorta from rabbits fed a cholesterol-enriched diet and stained with Sudan IV have been used during the development of this procedure. Sudan IV stains atherosclerotic lesions red and leaves healthy tissue with a pink tint.

A detailed discussion of the image analysis technique was proved in a previous interim report (4). In brief the television image processor used is integrated with an array processor and a Digital Equipment Corporation PDP-9 mini-computer to preprocess and threshold color television signals produced by viewing the photographic color image of sudanophilia on an arterial luminal surface specimen. The computer produces an exact contour map representing the area of healthy and diseased tissue. A statistical analysis may be made of the processed arterial specimen photographs which contribute to that portion of the data base to be analyzed. This results in a composite contour map depicting the probability of sudanophilic lesions occurring at any location on the luminal surface. These statistical maps may be grouped by epidemiology, age, sex and so on, in order to facilitate an analysis of the natural history of atherosclerosis.

The image processor itself analyzes the color video signal, first in terms of color, or hue, and then in terms of brightness, or luminance. Upon preprocessing a red hue, the image of the opened, flattened, Sudan IV stained, necropsy specimen is electronically converted to a binary array whose only values represent red and non-red areas. The luminance of all non-red areas is nullified, permitting only the red, or sudanophilic, areas to be further processed. Upon thresholding on luminance, then the preprocessed image is converted again, this time to a series of binary arrays, each of which represents the arterial image at a present luminance level, i.e. each binary mapping assigns a value to each array element as either above the given brightness level or below it. All binary maps are then overlaid in proper registration to produce a contour plot where one contour interval represents a present change in luminance of sudanophilic areas.

The image array, regardless of the magnification of the photographic image, contains a maximum of 180 x 256 elements, each element or pixel processed and stored separately. As the photographic image

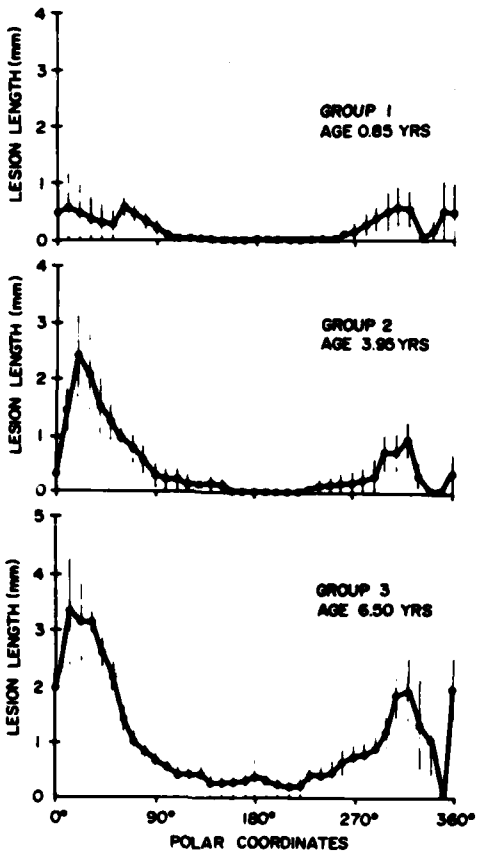

Fig. 1. Rectangular coordinate plot of length of coeliac sudanophilic lesions as a function of polar coordinate angle. Zero degrees and 360° are proximal (i.e. upstream) to the coeliac ostia and 180° is distal (i.e. downstream). Lesions develop initially in the proximal regions and then grow radially in the proximal region with some limited circumferential growth in the dorsal region with increasing age. (From Ref. 3)

magnification is increased (resulting in a smaller area included in the otherwise constant photograph dimensions), a proportionally higher resolution is achieved, since each pixel represents a smaller increment of surface area. Furthermore, these smaller, but highly magnified, partitions of the entire arterial image can be accurately and precisely reunited to restore the original image by joining the edges of the smaller, partitioned arrays stored in the computer memory.

To facilitate this restoration, as well as the subsequent registration of the overlaid binary maps and the statistical analysis, those array elements which correspond to arterial ostia, or geometric centers of ostia, are designated as landmarks. These landmarks then provide geometric indices at which the arrays of arterial photographic partitions are reunited, overlaid binary maps are registered, and a statistical transformation is performed.

The latter is used to overcome biological variability in arterial geometry through an approach in which each mapping undergoes a computerized statistical transformation into a standard arterial geometry, called the standard specimen. This transformation utilizes the ostial landmarks to construct linear transformation equations whose coefficients are unique to each specimen. These coefficients then are transformed so that the lines connecting adjacent landmarks coincide with the lines and landmarks of the standard specimen. These lines are selected so that they form adjacent triangles whose vertices are the ostial landmarks. In this way, each individual specimen's image array is distorted or stretched to conform to the standard specimen.

The result of this process is a contour map which conveys sudanophilic lesion information statistically. The information includes both surface area and location and is presented in terms of probability contours. The accuracy and presision of these quantification techniques are very high, limited only by the transduction properties of the luminance and chromaticity vidicons of the camera. Digital computer processing of the A/D converted camera signal is distortion free and exactly repeatable. This technique will have direct application to the study of the importance of a variety of factors in the atherogenic disease process. This includes environmental and occupation-related factors which might be of interest to the U.S. Air Force relative to its personnel.

III. WHOLEBODY VIBRATION AS A FACTOR IN DIET-INDUCED ATHEROSCLEROSIS

Studies have been conducted to evaluate the effect of low frequency, wholebody vibration on the formation of atherosclerotic lesions in the rabbit aorta. The initial chronic protion of this study has been carried out using four groups of rabbits. These groups

consisted of: 1) four vibrated rabbits fed a normal diet; 2) eight vibrated rabbits fed a 2% cholesterol enriched diet; 3) six non-vibrated rabbits fed a normal diet, and 4) eight non-vibrated rabbits fed a 2% cholesterol enriched diet. The test period for all animals was twenty-eight days. Before and after the test period, blood samples were obtained to determine the initial and final levels of serum cholesterol and triglycerides. During the twenty-eight day test period, the food intake of all animals was recorded. The vibrated animals were exposed to wholebody vibration by being restrained on an oscillating table for four hours per day, twenty-eight days in succession while being fully conscious. The table vibrated with a frequency of 5Hz and had a peak acceleration of 0.78g. The cholesterol feeding period for the vibrated animals coincided with the twenty-eight day period of exposure to vibration.

At the end of the twenty-eight day test period, the animals were anesthetized with nembutal (30 mg/kg) and sacrificed with the aortae being pressure fixed with a buffered formalin solution in situ and then removed. Following removal, the aortae were opened ventrally and stained with Sudan IV to indicate areas of sudanophilic lesions. The aortae were then photographed grossly and in close-up segment for the quantitative analysis techniques to be employed.

Stained periorificial lesion areas were quantified using polar coordinate mapping techniques by Cornhill and Roach (1) which was described in the previous section. The results from this study has shown that only rabbits fed the cholesterol enriched diet showed lesion staining after sacrifice. The vibrated animals had lesion staining in 87% of the ostia examined while the non-vibrated animals showed only 70% ostial staining with equivalent food consumption and equivalent final levels of serum cholesterol. This difference is significant for $p < 0.005$.

The polar coordinate mapping technique showed that all stained ostia consistantly exhibited distal lesions. The coronary and coeliac ostia also showed significant proximal lesions. Averaged polar coordinate maps for the coronary, intercostal and celiac ostia are presented in Figures 2 to 4. The mean lesion length and the lesion distribution were nearly identical for the vibrated and non-vibrated rabbits at the coronary and thoracic intercostal ostia locations. However, the larger abdominal ostia (celiac, renal and superior mesenteric ostia) of the vibrated rabbits have a greater mean lesion length and a greater extent of involvement around the ostia thus giving a different pattern of distribution.

In an effort to begin to understand mechanisms that may be involved in a wholebody vibration effect on the formation of atherosclerotic lesions, a study of the effect of acute vibration on arterial wall permeability was initiated. Intimal permeability was demonstrated using blue dyes (Evans and Trypan) which bind with serum albumin to form a stable complex. Areas of increased transport of

albumin may be observed as blue areas on the intimal surface of the rabbit aorta. Arterial intima are known to be very vulnerable to mechanical trauma and injuries that would initiate the development of arterial diseases. Using wholebody mechanical vibration it is indeed possible to enhance the albumin uptake and suggests that the enhanced blue uptake is a possible indication that intimal injury has been initiated.

Evans Blue and Trypan Blue were administered I.V. (1.25 ml/kg b.w. and 2 ml/kg b.w. respectively) in 15 rabbits. Five rabbits were vibrated longitudinally (along the cephalic-caudal axis) at a frequency of 10 Hz with an amplitude of 7 mm for a period of 1 to 5 hours. Three rabbits were vibrated transversely (displacement perpendicular to the cephalic-caudal axis) at the same frequency and for the same period of time. These two groups of rabbits were fully conscious during the period of vibration. Another group of 5 rabbits served as control, and they were not vibrated. In addition, 2 rabbits were vibrated longitudinally while being under deep anesthesia (nembutal; 30 mg/kg) for the purpose of direct blood pressure measurements. Indirect blood pressure measurements using the central ear artery was performed on all animals at various times. In this the central artery of the ear is occluded and the pressure needed for the occlusion is recorded as being the mean blood pressure. Thirty menutes after the end of the vibration period, the animals were anesthetized (nembutal; 30 mg/kg) and then sacrificed and the aortae were removed after a short pressure fixation. The aortae were trimmed, opened and examined for the accumulation of blue dye along the aorta.

In rabbits vibrated longitudinally, it was observed that the aorta had pronounced staining in the aortic arch and thoracic aorta and around the celiac and mesentaric ostia. In rabbits that were vibrated transversely, no enhanced accumulation of the dye was shown in any part of the aorta.

The blood pressure was significantly increased in the group vibrated longitudinally. This was confirmed both by the indirect and direct methods, and these preliminary results indicate a significant increase in pulse pressure from 35 to 60 mm Hg. This aspect alone deserves more investigation. No change in heart rate was recorded, but there was an apparent distortion of the electrocardiogram. In the group vibrated transversely, no change in blood pressure was observed using the indirect method.

The results of this present study indicate an influence of wholebody vibration on the uptake of albumin by the aortic endothelium. The increased blood pressure in the case of longitudinal vibration might account for the increased dye accumulation in the rabbits vibrated longitudinally. However, this study does not provide information as to which mechanism is involved in albumin entering the endothelium and as to how a vibration effect might be made manifest.

CORONARY OSTIA

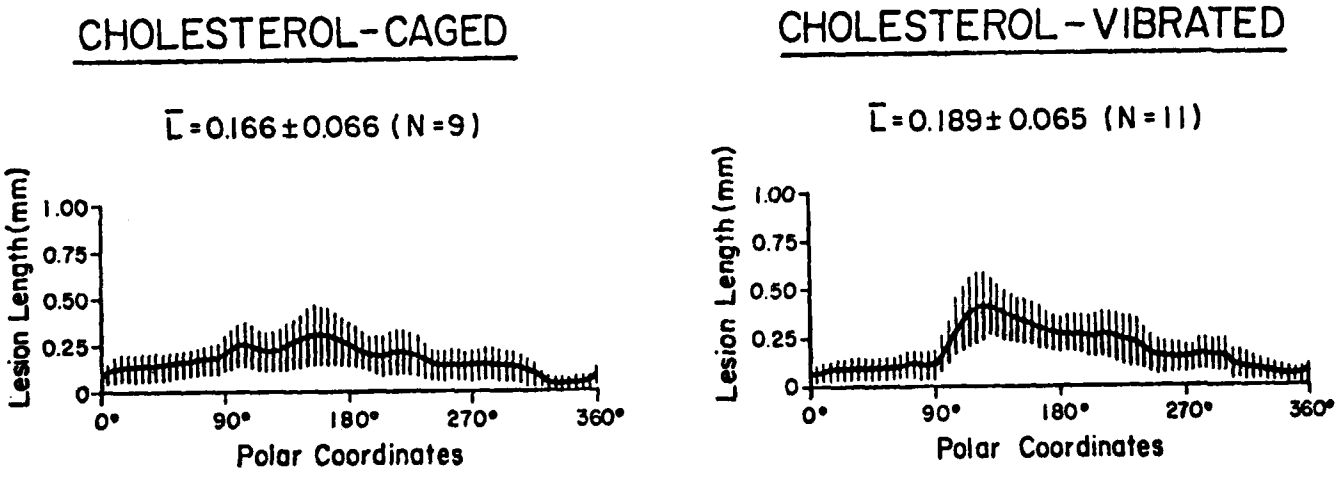

Figure 2. Polar coordinate mapping for the coronary ostia.

LEFT INTERCOSTAL OSTIA 1 TO 3

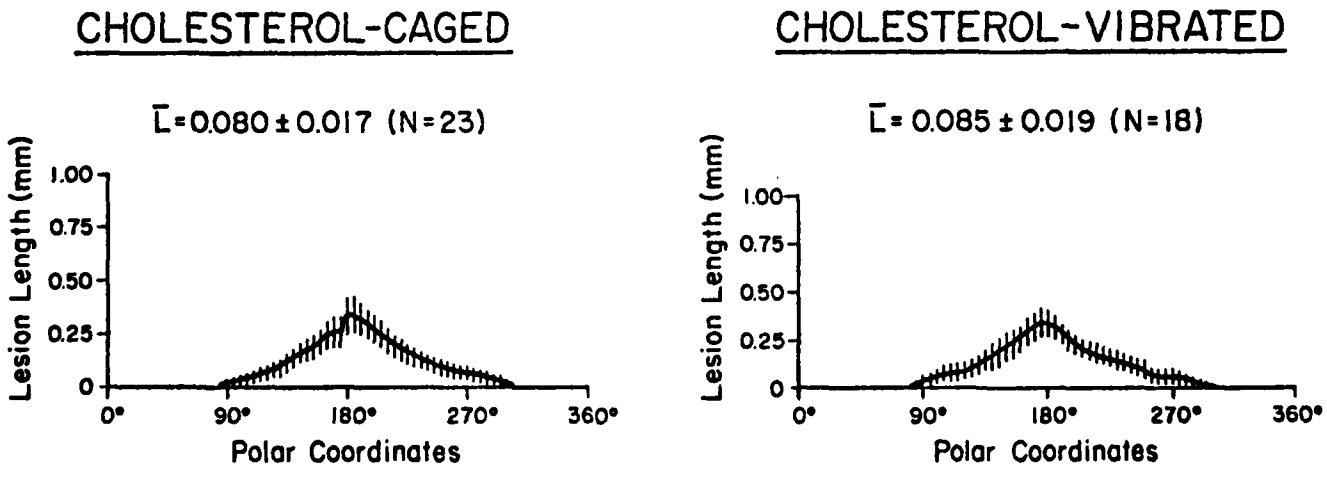

Figure 3. Polar coordinate mapping for the left intercostal ostia 1 to 3.

CELIAC OSTIA

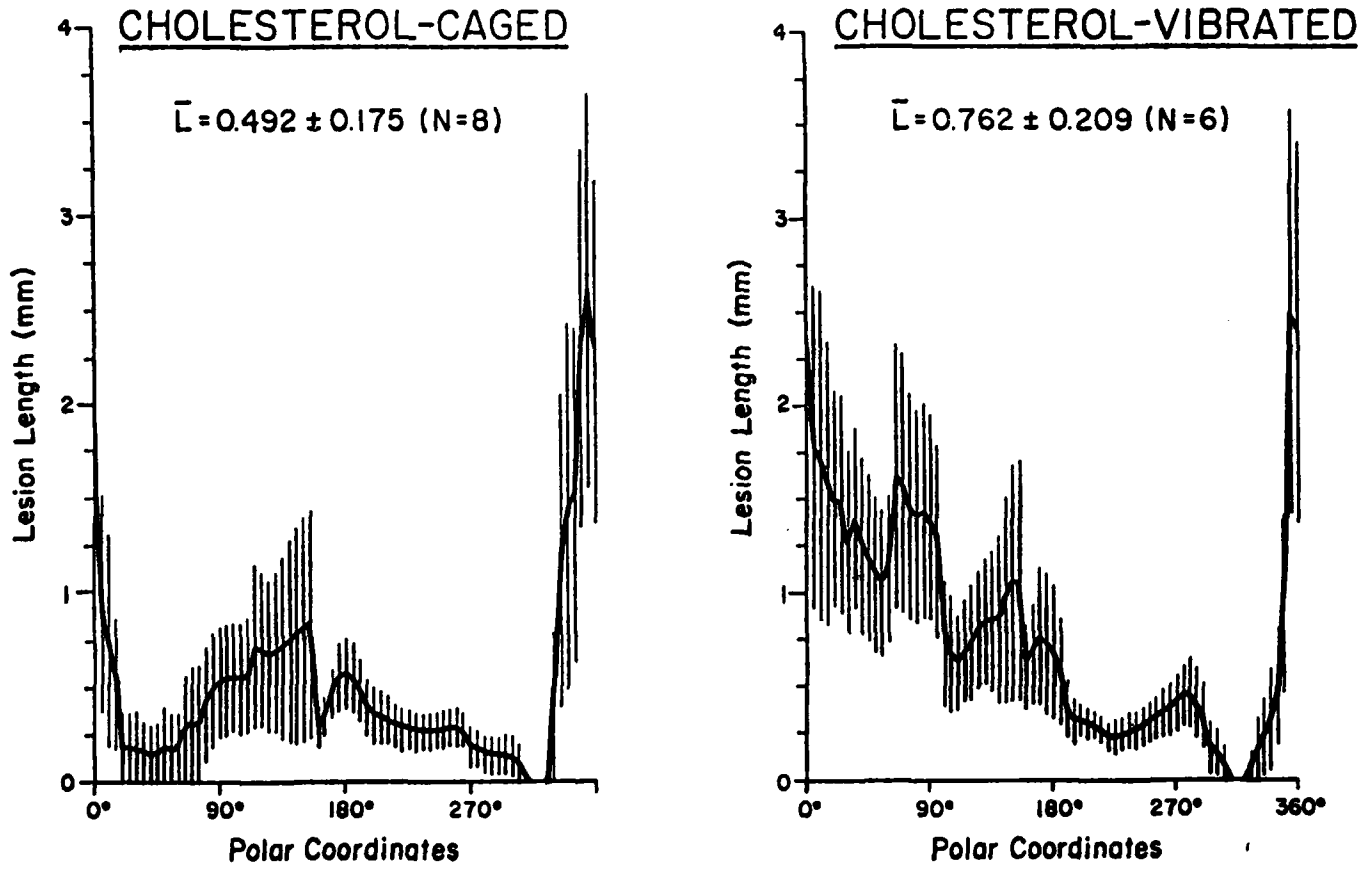

Figure 4. Polar coordinate mapping for celiac ostia.

It should be clearly recognized that these studies have been designed to determine only whether vibration is capable of altering aortic blue dye uptake. However, these results have lead the investigators to adopt a more critical approach in planning their experimental design, and it is now considered rather urgent to determine the effect of acute wholebody vibration before investing too much more effort in long term, chronic studies.

IV. SOCIAL ENVIRONMENT AS A FACTOR IN DIET-INDUCED ATHEROSCLEROSIS

The existence of a relationship between stress and cardiovascular disease comes from a variety of evidence implicating physical, emotional, and behavioral factors. These include recent findings linking coronary heart disease to prolonged emotional stress, behavior patterns and changing life events, as well as studies suggesting that stress associated both with our modern industrialized society and with specific professional roles result in a greater incidence of both hypertension and coronary heart disease. There are also data from experimental animal studies linking psychosocial disruption to pathological changes in the cardiovascular system. These have included studies where states of severe emotional disturbances in animals were produced using various negative stressors such as sound and electrical shock. To our knowledge, however, there have not been any studies which investigated the effects of possible positive factors.

In this project a series of studies designed to investigate the influence of social environment on diet-induced atherosclerosis in rabbits has been carried out. The social environment employed was one of tactile contact and caring in which the animals were individually petted, held, touched, fed, talked to and played with. The social environment focused on here is thus that associated with the interaction between laboratory animals and an experimenter.

Three experimental groups were studied (Groups A, B, and D) where the animals experienced the experimental tactile contact and caring environment described in the preceding paragraph. There also were two control groups (C and E) in which the animals received normal laboratory animal care. Group A was studied without control during late 1977. It was one in which the actual intent was not to investigate any influence of social environment; in fact, the expressed purpose of Group A was for it to be a control for a parallel group on the identical diet, but undergoing pharmacological intervention. However, the serendipitous results obtained for Group A, although initially considered anamalous, upon further analysis led us to conduct two additional studies. One of these was carried out in early 1978 and involved experimental Group B and control Group C. The other was carried out in late 1978. It involved experimental Group D and control Group E.

It should be noted that the essence of the experimental environment studied here was to establish a pet, one-to-one relationship between each animal and the experimenter (M.J.L.). This was done through a protocol which on a daily basis included an early morning, one-half hour long visit during which each animal was handled, touched, stroked, talked to and played with; an hour long feeding period during which each animal was also touched and talked to; and a number of short, 5-minute visits during the day with a variety of contact and communication. Through this daily process the animals quickly learned to recognize the experimenter, and when present, many actually sought this individual's personal attention. The animals were left alone for a ten-hour period each night.

The animals used in this study were young male New Zealand white rabbits weighing approximately 2 kg at the start of the experimental period. Upon receiving the animals, the animals were separated and subjected to a 2-week adaptation period before being exposed to a 2 percent cholesterol diet. During this period, food (rabbit chow) and water were supplied ad libitum and their daily food intake was recorded. It is here that the animals and the experimenter first became acquainted with each other. It is important to note that all experiments were carried out by the same experimenter who followed the same protocol for animal caring.

After the adaptation period, the animals were fed a regular rabbit diet supplemented with 2 percent cholesterol (ICN Nutritional Biochemicals, Cleveland, Ohio 44128). Daily food intake for each animal was recorded. Animals from Groups A, B, and C were sacrificed after 5 weeks and the animals from Groups D and E after 6 weeks. At weekly intervals body weight was measured. For blood chemistry analysis, blood samples were withdrawn after a 12-hour fast and total serum cholesterol levels were determined at weekly intervals. Animals from Group A were not fasted. Indirect blood pressure was determined at weekly intervals using an ear cuff in Groups A, D, and E. In Groups D and E direct blood pressure measurements were also obtained at sacrifice via left carotid artery catherization. In addition the heart rate was determined in these animals.

At the end of the experimental period, the animals were anesthetized (nembutal; 30 mg/kg) and the thorax was opened to expose the heart. A cannula was inserted into the left ventricle, advanced to the level of the ascending aorta, and secured with a purse string. The aorta was perfused <u>in situ</u> at physiological pressure (95 mm Hg) with 10% isotonic formalin for 3 hours. At the end of this period, the entire aorta was excised and opened from the dorsal aspect. The specimens were stained with Sudan IV and were pinned out for subsequent photography. Sudanophilia, as evidenced by the darker regions corresponding to the uptake of Sudan IV, was noted in specimens from all groups. However, the presence of sudanophilia was much more striking in Groups C and E, and based on visual observations, there appears to be a

significant decrease in aortic surface sudanophilic involvement for those animals experiencing the experimental social environment.

This observation is borne out by the more quantitative analysis of data presented by Figure 5. Here the percent of total aortic surface area exhibiting sudanophilia is presented for each of five groups.. As may be seen, the experimental groups (A, B, and D) have a percent surface area involvement which is reduced by more than 60 percent compared to that for the control groups (C and E). A statistical analysis has been carried out for the results from Group B compared to its paired control, Group C, and from Group D compared to its control, Group E. This indicated the difference in aortic sudanophilia to be significant (for B-C, $p = 0.015$; for D-E, $p = 0.034$).

For paired groups B and C, the mean final serum cholesterol levels were 1527 ± 125 (S.E.M.) and 1426 ± 298 mg/dl respectively. This difference was not statistically significant ($p = 0.50$). For paired Groups D and E, the mean final serum cholesterol levels were 1980 ± 419 and 1881 ± 214 mg/dl respectively. Here again the difference is not statistically significant ($p = 0.65$).

As noted earlier both indirect and direct measurements of blood pressure were made, although not in all groups. For Groups D and E the blood pressure levels were found to be 72 ± 9 and 74 ± 4 mm Hg respectively. This difference is not statistically significant ($p = 0.76$). Heart rate measurements were also carried out in Groups D and E with values of 191 ± 35 and 219 ± 19 beats per minute being obtained. This difference is also not statistically significant ($p = 0.15$).

This series of studies indicates that social environment, to be specific the tactile contact and caring experimental environment provided here, has a dramatic effect on diet-induced aortic atherosclerosis in rabbits. Having in effect conducted three separate experiments, we feel that our results are reproducible in spite of the fact that the social environment employed here is not as quantifiable as those protocols used where physical or biochemical interventions are studied. However, there are obvious questions relating to the possible mechanisms that might be involved in this demonstrated effect of a particular social environment on atherogenesis. With regard to this, it is clear that measurements of the effect of social environment on blood hormonal levels, arterial wall permeability, endothelial regeneration rates, as well as a myriad of other properties would all be important.

There also is a very important question raised by these results as to whether it is possible that data obtained in the past from different laboratories for ostensibly the same experiment might be contradictory solely because of a difference in social/psychological environment? If this is possible, and we believe it is, then it also

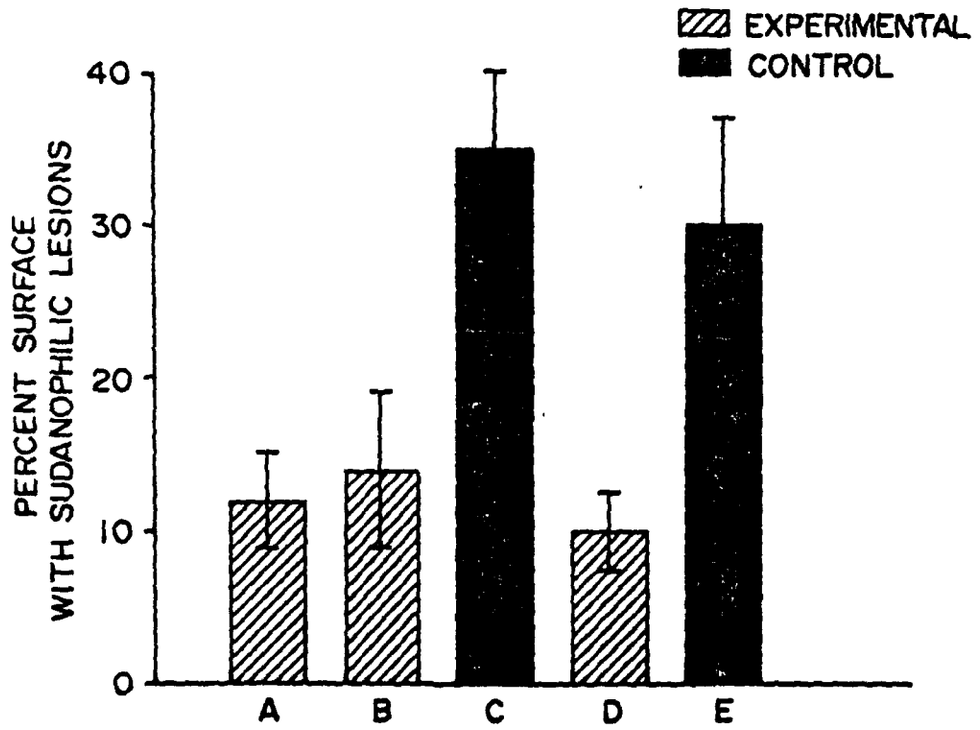

Figure 5. Average percent of aortic surface area exhibiting sudanophilia for both experimental and control groups; bars show standard error from the mean.

would be possible to explain in this way anomalous results within a single laboratory. For example, the differences observed here are of the same or greater magnitude as those reported in many instances where the effect of a particular intervention on atherosclerosis was investigated using the hypercholesterolemic rabbit as an animal model. If no attention was given to social environment in the protocol used to study a particular intervention, what was the real effect observed? Clearly, more needs to be known about social environment in studies of disease using animal models. If nothing else, these results suggest that, in specifying the protocol for an animal study, consideration should be given to the inclusion of social/psychological environmental factors.

V. THE STUDY OF THE ENDOTHELIUM

Endothelial cell nuclei have been shown to respond to hemodynamic stresses by altering their orientation and geometry (5). It is the purpose of the studies described in this section, to develop techniques of preparing the endothelium for examination, to develop techniques of analyzing quantitatively endothelial cell morphology, and finally, to apply these techniques to determine how mechanical forces affect the arterial endothelium.

A technique of studying the vascular endothelium was developed recently in our laboratory. This technique is described in detail in our recent publication (6) which is enclosed with this report. In brief, animals are anesthetized with Nembutal (i.v. 30 mg/kg), heparinized (1000 U/kg body weight), and the heart is exposed by means of a sternal split, resulting in cardiac fibrillation and animal death. A cannula (5 mm) is introduced into the left ventricle and is secured with a purse-string. The aorta is flushed with fresh glucose solution (5% dextrose) and then flushed with the same volumes of glucose and saline (0.9% NaCl). The above perfusions are freshly prepared and filtered with milipore filter (0.3 um). The perfusates are all kept at 38°C. The casting material [Batson's 17 (Polysciences, Warrington, PA 18976)], diluted with methly methacrylate, is then infused into the vessel at constant pressure.

After the plastic has set completely (the material cures in 45 minutes, but the quality of the cast is greatly improved by leaving the cast in the animal overnight), the aorta and cast are excised and placed in a saturated solution of sodium hydroxide for a 12-hour period during which the cast is rinsed frequently in distilled water. If the tissue is not completely dissolved after 12 hours, the cast is then placed in a milder solution of Potassium Hydroxide (approximately 2 M) for one day. The imprints of the aortic endothelial cells on the vascular casts are examined using a Nikon-Biophot microscope. Examples of the quality of these photomicrographs are seen in Figure 6 and in our published work (6,7).

In order to make quantitative statements about endothelial cell morphology, a computerized system has been developed which describes the quantitative morphology of endothelial cells in terms of 8 calculated parameters (area, perimeter, length, width, angle of orientation, width:length ratio, axis intersection ratio and shape index).

Photomicrographs with the imprints of 15-50 endothelial cells were analyzed quantitatively using computer programs written specifically to determine the morphological parameters (Figure 7). The outlines of all cells were digitized using a Bendix Datagrid Digitizer. In brief, the digitization consisted of entering an identification code, a scale factor and the direction of blood flow. The boundaries of the individual endothelial cells were then traced in succession and the digital information stored on magnetic tape. Between 1000 and 1500 data points, depending on cell size and the rapidity of digitization, were used to define the border of each cell. The magnetic tape, which contains the digitized information describing the cell boundaries, was then submitted for processing on a digital computer.

The computer algorithms, which have been developed to analyze these data, calculate 8 parameters which describe quantitatively the morphology of the endothelial cells (Figure 7). The definitions, methods of calculation and examples of the way in which these parameters describe cell morphology appear in our recently published paper (7).

A quantitative study of the en face size and shape of rabbit endothelial cells from the ventral mid-thoracic and ventral infrarenal abdominal aorta has been carried out in 6 rabbits. Photomicrographs were taken from vascular casts of the rabbit aorta and the endothelial cell outlines were analyzed quantitatively using a digitizer and digital computer. The morphology of the endothelial cells was described using the 8 previously noted calculated parameters. The endothelial cells in both locations had the same surface area ($p < 0.30$); however, the cells in the abdominal aorta were longer ($p < 0.01$) and narrower ($p < 0.01$) than those in the thoracic aorta. This fact is reflected by the smaller value for the shape index and width:length ration in the abdominal aorta ($p < 0.01$). Cells in both the thoracic and abdominal aorta were aligned with the flow direction.

Photomicrographs for the thoracic and abdominal aorta are seen in Figure 6. The distrigution of the data is shown in Figures 8 and 9 and the tabulated results appear in Table 1. Further details may be found in our recently published paper (7).

In summary a reproducible method has been presented which accurately describes the quantitative en face cellular morphology of the arterial endothelium. The use of non-parametric statistics allows one to make statistical statements concerning the differences in quantitative morphology between populations of cells. In a

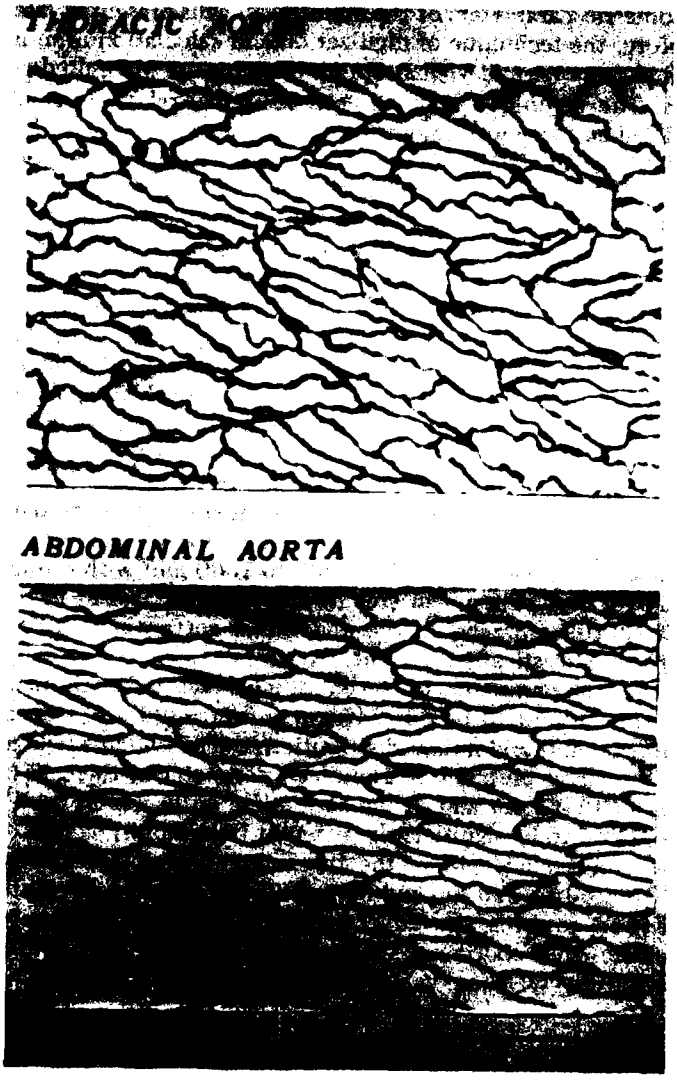

Fig. 6. Photomicrographs of endothelial cell outlines from the thoracic and abdominal region of the aortic vascular cast. Cell borders appear as dark lines and the imprints of the cell nuclei are visible in some cells.

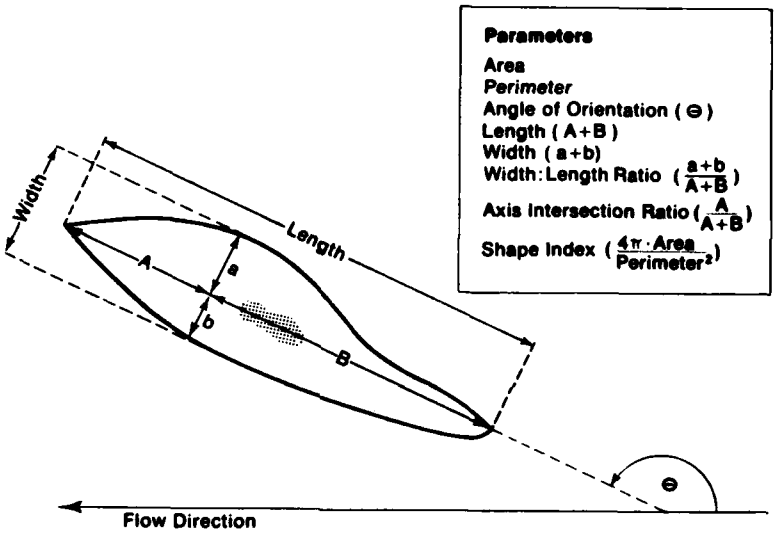

Fig. 7. Scheme of the morphometric parameters calculated for each endothelial cell.

TABLE 1

ENDOTHELIAL CELL MORPHOLOGY - RABBIT AORTA

Mean value ± SEM

	Thoracic aorta	Abdominal aorta	P-value
Number of cells[a]	260	333	--
Area $(\mu m)^2$	789 ± 109	729 ± 80	n.s.
Perimeter (μm)	140 ± 11	164 ± 17	0.005
Length (μm)	63 ± 5	76 ± 10	0.003
Width (μm)	19 ± 3	15 ± 1	0.01
Angle of orientation	4° ± 11	5° ± 10	n.s.
Width:length ratio	0.29 ± 0.04	0.20 ± 0.04	0.003
Axis intersection ratio	0.49 ± 0.03	0.57 ± 0.02	0.04
Shape index	0.50 ± 0.05	0.36 ± 0.07	0.002

[a]From 6 rabbits.

n.s. = $P > 0.05$

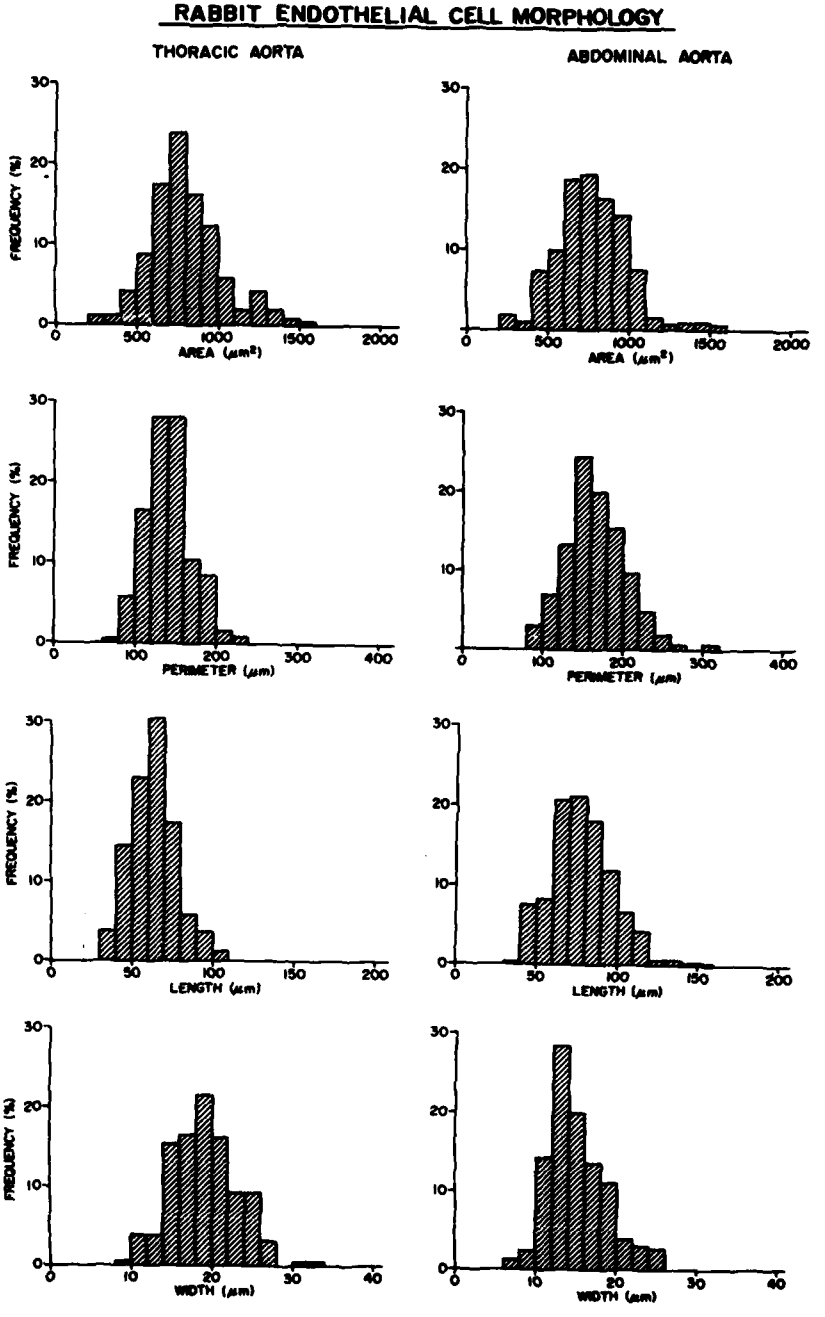

Fig. 8. Histograms for the observed values of endothelial cell area, perimeter, length and width for cells from the thoracic (n=260) and abdominal (n=333) rabbit aorta.

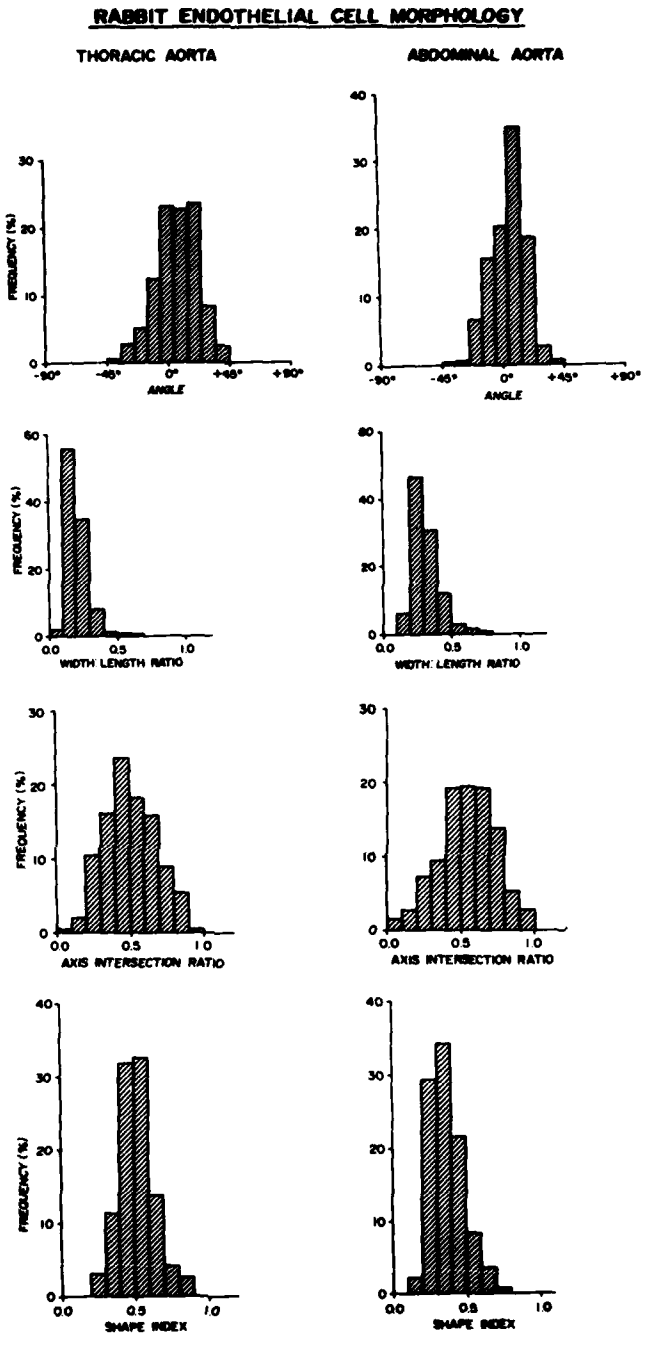

Fig. 9. Histograms for the observed values of endothelial cells angle of orientation, width:length ratio, axis intersection ratio and shape index for cells from the thoracic (n=260) and abdominal (n=333) rabbit aorta.

demonstration of the efficacy of the technique, the cells in the rabbit abdominal aorta were found to be longer and narrower than the corresponding cells in the thoracic aorta while having the same surface area and both being oriented with the direction of blood flow. The technique has numerous applications in the quantitative study of the arterial endothelium and its response to injury.

VI. CONCLUDING DISCUSSION

The techniques which have been described in the preceeding sections are ones which lend themselves to the quantitative study of the cardiovascular system and in particular to the study of atherogenesis and factors which may be associated with the disease process. The motivation for developing these techniques has been to better understand what role, if any, high intensity, intermediate duration, low-frequency wholebody vibration may play in a specific cardiovascular disease process. However, these techniques have a wider range of applicability and our interest now includes a whole variety of interventions which might be mitigating factors in the atherogenic process. One such factor is psychological stress. The preliminary results in our laboratory which were discussed in Section IV indicate that the positive emotional stress effected by placing animals in a condition of tactile contact, caring and reassurance can drastically alter the animal's responses to a high cholesterol diet. These studies need to be pursued further; however, it is the entire range of environmental stresses including both physical and psychological stresses to which our interest is now turning and to which the techniques discussed herein can be applied.

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