Myocardial Lysosomes in Experimental Atrial Septal Defects

By Charles A. Kottmeier, M.D., and Myron W. Wheat, Jr., M.D.

ABSTRACT

Preliminary observations in patients undergoing open-heart surgery have suggested that the lysosomes in the right atrium are increased in patients with atrial septal defects. This increase in lysosomes per myocardial cell appears to be directly related to the size of the left to right shunt. The following experiments were performed to see if similar findings could be produced in an experimental model. Atrial septal defects were produced in seven dogs. The biopsy specimens were prepared for ultrastructural examination and viewed with an electron microscope. Lysosomes were counted in representative sections and an estimate of the lysosomes per square micron of heart tissue obtained. The number of lysosomes per square micron of myocardium increased significantly following the creation of atrial septal defects in dogs, with the most marked increase occurring in the right ventricle. Several small atrial septal defects closed spontaneously, as proved by cardiac catheterization, and in these animals the elevated lysosome counts present initially returned toward normal after obliteration of the left to right shunt at the atrial level. Control dogs showed no significant increase in myocardial lysosomes over the 18-month period despite repeated thoracotomies and myocardial biopsy. These studies add further evidence to support the role of the lysosome as an important intracellular organelle which is related to cellular stress.

ADDITIONAL KEY WORDS
chronically stressed myocardium lysosomal activity dog left to right shunt at the atrial level intracellular organelle

Since 1955, when de Duve postulated the existence of the lysosome (1, 2), there have been many attempts to characterize the activity of this small intracellular organelle (3). Many of the enzymes contained in the lysome are well known (3, 4). Lysosomal activity in liver and kidney has been investigated following the administration of toxic substances (5-8), and this research has led to the suggestion that one function of the lyso-
would confirm the previous observations in patients and further support the role of the lysosome as an integral part of the intracellular metabolic process.

**Methods and Materials**

Mongrel dogs, 12 to 20 kg, were anesthetized with pentobarbital, placed on a positive pressure ventilator, and myocardial biopsies of the walls of the four heart chambers were taken through an open thoracotomy. Atrial wall biopsy sites were from the base of each appendage. Ventricular biopsies were taken approximately 2.0 cm from the interventricular septum midway between the apex and great vessels. After the control biopsies, an atrial septal defect was created by the closed technique of Blalock-Hanlon (11) in 4 dogs or by direct, open visualization and removal of a large segment of the interatrial septum using inflow occlusion in 2 dogs. The period of inflow occlusion was never longer than 70 sec. At the time of surgery, the atrial septal segments removed were 1.0 to 1.5 cm diam in the Blalock-Hanlon procedures, and up to 2.5 cm diam in the inflow occlusion procedures. In 2 control animals, open thoracotomy with myocardial biopsies was performed but no atrial septal defect was created. At intervals of 1, 3, 6, 12, and 18 months later, repeat biopsies were taken by open thoracotomy.

The tissues obtained by biopsy were immediately diced in 3% glutaraldehyde buffered to pH 7.4 with 0.2M sodium cacodylate buffer with sucrose (12) and fixed at 0 to 4°C for 1 hr. They were then rinsed and stored in sodium cacodylate buffer (pH 7.4). When further processing was done, the specimens were postfixed in phosphate buffered osmium (OsO4) tetroxide (pH 7.4) (13) at 0 to 4°C for 1 hr. Dehydration was carried out in graded concentrations of ethyl alcohol and embedding was performed in a 7:3 mixture of Epon 812 or Araldite 502 (14). The tissues were sectioned on Porter-Blum MT-1 and MT-2 ultramicrotomes using diamond knives and mounted on 300 mesh copper grids with colloidin film and carbon coating. The grids were stained with uranyl acetate and lead citrate (15, 16) and viewed in an Akashi Tronoscope TRS50E1 or an Hitachi Hu 11-B electron microscope.

Lysosomes were counted on a morphological basis after having first established that organelles of this morphological description in both human

![Changes in lysosomes in right atrium and right and left ventricles in dogs with Blalock-Hanlon atrial septal defects which closed, Blalock-Hanlon atrial septal defects which remained open (upper graphs), and large, 2.0 to 2.5 cm atrial septal defects which remained open (lower graphs), as compared to controls.](image)
and canine myocardium contained acid phosphatase. The method for the localization of acid phosphatase was the same as previously reported in studies on patient material (9).

In the present studies, lysosomes were counted on the fluorescent screen at magnifications of from 6,000 to 8,000x, and verified with occasional representative electron micrographs (see figures). The number of lysosomes in each grid square was counted. Only one square of serial sections was counted to avoid counting the same lysosome twice. Each grid square is 50 μ². Only grid squares containing exclusively muscle cells were counted. Twenty-five to 50 grid squares from one or more blocks of tissue were counted to constitute a representative sampling of each specimen and the average number of lysosomes per 100 μ² in each group were plotted against the time after initial biopsy and creation of the atrial septal defect.

In the 4 dogs with Blalock-Hanlon atrial septal defects, right heart catheterizations were performed after the 6-month interval biopsy. Dye dilution curves (17) using indocyanine green (Cardio-green) were obtained with the injection sites in the pulmonary artery, right ventricle, high, middle, and low right atrium and the superior and inferior venae cavae. A retrograde femoral arterial catheter for sampling was passed well up into the aorta. Location of the catheter on the right side of the heart was determined by pressure tracing and fluoroscopy. No cardiac catheterizations were performed on control dogs or those with defects created by the inflow occlusion technique. Dogs which died either during or after later biopsies were necropsied and the defects were examined to correlate the anatomy with the experimental data.

The data were analyzed using the Dunnett multiple comparison method which seems to be the most reliable and accurate analysis method available for our experiments (18).

Results

In the 2 dogs with atrial septal defects created by the Blalock-Hanlon technique, there was a significant (P = .05), greater than two-fold increase in lysosomes in only the right ventricle at the 1-month biopsy. Lysosome counts from biopsies at 3, 6, and 12 months
after the initial procedure showed no statistically significant difference from control counts. After 6 months cardiac catheterization in these 2 dogs showed no left to right shunts, indicating that the atrial septal defects had healed. There were no statistically significant changes in the lysosome counts in biopsies obtained from the other chambers in these animals (Fig. 1).

In 2 dogs with Blalock-Hanlon defects, which had 10 to 12% left to right shunts at the atrial level after the 6-month biopsy documented by dye curves, the myocardial lysosomes showed a three to fourfold persistent increase in the right atrium, and right and left ventricles (Fig. 1). In the right atrium, all changes were statistically significant \( (P = .01) \) at the 1-month and subsequent biopsies. In the right ventricle, the change in lysosomes was significant \( (P = .05) \) at the 1-month interval, and subsequent biopsies showed changes of greater significance \( (P = .01) \) at the 3-, 6-, and 12-month intervals. In the left ventricle, changes in lysosomes were significant \( (P = .05) \) in all interval biopsies.

A fifth dog was initially included in this study but died of ventricular fibrillation as the incision was being closed after the 3-month interval biopsy. Necropsy revealed definite partial closure of the septal defect, with a 3 mm margin of scar tissue extending from the margins of the resected septum. The original defect in this animal was 1.0 cm diam.

In the 2 animals having 2.0 to 2.5 cm defects created by the open inflow occlusion technique, the number of lysosomes seen was as much as six times that seen in the control

**Figure 3**

Electron micrograph of a 3-month biopsy from the right ventricle showing vacuolated lysosomes in clumps dispersed between mitochondria. EC = ergastoplasm, others as in Figure 2. Original magnification \( \times 9,000 \).
MYOCARDIAL LYSOSOMES

(Fig. 1). These elevations in lysosome counts persisted through the 18-month period after the initial procedure. The most marked increases in lysosomes were in the ventricles where they ranged as high as 20 lysosomes per 100 μ² compared to the control levels of 3 lysosomes per 100 μ² (Fig. 1). In the right atrium the changes in the number of lysosomes were significant (P = .01) for all experimental biopsies as were the changes significant in the right ventricle (P = .01). The changes in lysosomes in the left ventricle at the 1-month biopsy were less but still statistically significant (P = .05). There were more significant differences in subsequent biopsies (P = .01) at 3, 6, and 18 months after the initial procedure.

Control animals showed no increases in the number of lysosomes for 18 months despite repeated thoracotomies and biopsies. In our studies of the lysosome, their appearance has been strikingly similar from sample to sample (see Figs. 4, 5, and 6). They are usually oval or elongated oval, from 4 to 12 μ average diam, and are bounded by a unit membrane. They contain a coarsely granular, moderately dense osmiophilic material which surrounds one or more irregular, less dense areas which are frequently near the center of the lysosome. The lysosomes also contain relatively large globules of medium osmiophilia which are surrounded by a well defined dense membrane and contain clear, sharply bounded spaces within

![Figure 4](http://circres.ahajournals.org/lookup/suppl/doi:10.1161/01.RES.21.4.21.1.html/-/DC1/FIG4.jpg)

**FIGURE 4**

Electron micrograph of a 6-month biopsy from the right ventricle showing a small clump of densely vacuolated lysosomes. Mf = myofibrils, others as in Figure 2. Original magnification X 13,000.

Circulation Research, Vol. XXI, July 1967
them. The large globules of medium osmiophilia are most often adjacent to the outer wall of the main body of the lysosomes and frequently show some concavity along one edge.

In the present studies not only the quantity of lysosomes changed but there appeared to be an additional qualitative change. The structure of the lysosomes changed with the persistence of the left to right shunt. Initially, lysosomes were seen in small numbers, and were small in size with small and infrequent vacuoles (Fig. 2). During later stages, following the creation of atrial septal defects, the lysosomes increased in over-all size, in the numbers of vacuoles within each lysosome and in the size and complexity of the vacuoles themselves (Figs. 3 and 4). Clumps of lysosomes were frequently seen in biopsies from the hearts with larger defects (Figs. 3 and 5). These clumps of lysosomes contained many large lysosomes as well as what appeared to be small developing "primary" or "prelysosome" forms (Fig. 5, PL). These lysosomes have the appearance of the organelles described by Jamieson and Palade (19) as occurring most frequently in the atrial tissue of smaller animals, such as the

FIGURE 5

Electron micrograph of a 12-month biopsy from the right ventricle showing multiple clumps of vacuolated and nonvacuolated lysosomes found consistently in biopsies taken at this interval after creation of a large atrial septal defect. Small prelysosome or primary lysosome forms are seen; they were shown not to be portions of larger lysosomes by evaluating serial sections. PL = primary lysosomes, others as in Figure 2. Original magnification X 5,000.
rat and dog. However, they were present in both the atrial and ventricular muscle of our animals and therefore are not specifically related to one heart chamber or the other. The distribution of these clumps of lysosomes did not follow a specific pattern but many were noted near the nuclear poles. There seemed to be no orientation or localization of lysosomes specifically related to intercalated discs.

In control specimens, lysosomes were fairly evenly dispersed throughout the sarcoplasm of the cell, interspersed among the myofibrils with the mitochondria and other intercellular organelles. The lysosomes seemed to be more prominent at the nuclear poles than any other single area. In all representative electron micrographs taken, there appears to be a constant anatomical association of lysosomes with ergastoplasm and Golgi apparatus. This would be expected if Novikoff's postulation of the origin of lysosomes from the Golgi apparatus is correct (20).

Discussion

In this series of experiments, each experimental dog served as his own control in addition to two animals that were run as strict parallel controls. It was not the original intent of the experiment to have spontaneous closure of some of the defects. However, having two defects close spontaneously showed that the stress of a left to right shunt produced an increase in myocardial lysosomes which was followed by a return toward control levels once the stress was no longer present (Fig. 1). The opposite effect was also seen in that persistence of the atrial septal defect with the continued stress of a left to right shunt produced a continued elevation in the number of myocardial lysosomes and, presumably, a sustained increase in myocardial lysosomal activity.

Both the right and left ventricles had an increased number of lysosomes during prolonged stress. At first it would seem unexpected to find increased numbers of lysosomes in the left ventricle since the left to right shunt in atrial septal defects bypasses the left ventricle. However, although the heart does have a right and a left side, it functions as an over-all, single organ. A given hemodynamic lesion may affect one side more than the other, but in a chronic situation, particularly, intracellular manifestations of increased work by any part of the heart should be present to some extent in all myocardial cells.

In these experiments, chronic stress to the myocardium was applied by surgically creating left to right shunts of varying degrees. The results indicate that there is a positive correlation between the size and persistence of the left to right shunt and the number of lysosomes. The morphologic changes in the lysosomes seen in the more chronically stressed myocardium suggest increased enzymatic activity, perhaps as a response to increased metabolic products and autolytic debris within the myocardial cells. The mechanisms of this phenomenon are not explained here, but the lysosome, by its definite quantitative as well as qualitative change, is undoubtedly involved in the intracellular response to stress, in this instance primarily metabolic. The response and activity of the lysosome to foreign body and bacterial intracellular invasion has been previously well documented (5-8). The present studies confirm our earlier findings obtained in human myocardium chronically stressed by congenital and acquired heart disease (9, 10). The right atrium was the only myocardial tissue previously evaluated in the human studies. However, on the basis of the results in these experiments, it appears that an increase in the number of myocardial lysosomes can be expected in those cells subjected to increased metabolic demands. The greatest change in the number of lysosomes in these experiments was in the chamber subjected to the greatest stress, the right ventricle. How close the correlation is between the degree of stress and the elevation in lysosome count cannot be answered from our present data. Although a lysosomal/myocardial index as a possible indicator of the degree of severity of heart disease would be a helpful tool, we cannot substantiate more than a general relationship at this time.
Acknowledgment

This is to acknowledge the technical assistance of Mr. James Smith, Mrs. Mary Taylor and Mrs. Marcia Degenhardt.

References


Myocardial Lysosomes in Experimental Atrial Septal Defects
CHARLES A. KOTTMEIER and MYRON W. WHEAT, Jr.

doi: 10.1161/01.RES.21.1.17

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231 Copyright © 1967 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/21/1/17

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/