STUDIES IN EXPERIMENTAL ENDOCARDITIS

I. Production of Valvular Lesions by Mechanisms Not Involving Infection or Sensitivity Factors

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Many experimental studies have implicated allergic sensitization or local infectious mechanisms in the formation of valvular vegetations. Though a number of organisms have been used, staphylococci and streptococci have been employed most often. Jones and Carter [1] have gathered the references demonstrating the role of allergy, streptococci or both; their review also noted that valvular lesions followed anaphylactic shock and injections of whole or fractionated horse, bovine, duck or pig serums and streptococcus toxin. Thomas, Brunson and Smith [2] and Stetson [3] used meningococcus toxin to induce a local Shwartzman phenomenon in the valve. Strehler [4] utilized anti-aorta serum.

It is difficult to invoke the phenomenon of sensitivity to explain valvular lesions that follow a single injection of an antigen, particularly when they occur rapidly and before allergic sensitivity or antibody can be demonstrated.[2,3] Jones and Carter [1] noted this fact and also produced lesions with tested nonallergenic material, such as neutral polysaccharide, vitreous humor and gastric mucin. Rinehart and Mettier [5] observed that scurvy played a role in the production of valvular lesions. There was no evidence that sensitivity was instrumental in the appearance of valvular vegetations following such nonspecific forms of stress as high altitude,[6] arteriovenous shunts,[7,8] pitressin [9] or papain and amphetamine sulfate.[10]

The high incidence of nonbacterial vegetations with many sensitizing substances and nonantigenic forms of stress would seem to exclude local infection as the common denominator in the induction of their initial valvular lesions. These considerations, in conjunction with the frequent occurrence of vegetations of this nature on human valves [11,12] without evidence of infection or antigen-antibody mechanisms, as in "terminal endocarditis" in patients with uremia or cachexia, and the frequent

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transitions encountered in all forms of endocarditis,[13] favor the hypothesis that local sensitivity or infection is not generally a factor in the evolution of the early phases of valvular vegetations.[14]

The present series of experiments was undertaken to develop methods of producing vegetations and investigating their early stages. Rats were subjected to various types of nonspecific stress for these purposes.

Material and Methods

Adult male and female rats of the Sprague-Dawley strain, weighing approximately 200 gm., were used. These were maintained on Purina Rat Chow and water, unless otherwise stated.

The physiologic stresses cited below were purposefully selected to avoid the factors of infection, sensitization and immunization. In each experimental group there were usually 5 control animals kept under identical conditions without the addition of the particular stress. In addition, there was a special control group of 56 rats maintained with all forms of stress reduced to a minimum.

Normal Rats

The occurrence of some alterations in occasional parallel control rats prompted a more detailed investigation of a larger series of normal rats kept under conditions of minimum stress. These animals were isolated from 1 to 12 weeks in individual cages and kept in an air-conditioned room at 72° F. and 50 per cent humidity. The 56 rats handled in this fashion were of different ages and were grouped by weight as follows: males: 5 less than 150 gm.; 16, 151 to 300 gm.; 11, more than 300 gm.; females: 6 less than 150 gm.; 18 between 151 and 500 gm. These served to establish the range of spontaneous lesions in the valves of this strain of rats at different ages, sizes and sexes.

Nonspecific Forms of Stress

1. Tumbling. Sixteen rats were placed in a tumbling machine 3 times a week. Two hundred tumblings were administered initially; there were increments of 100 until 1,000 tumblings per session. The rats were sacrificed after 4 to 35 such treatments.

2. Exposure to cold. Sixteen rats were placed in a cold room at 4° C., 4 hours a day, 4 days a week. The animals were sacrificed after 36 to 131 exposures.

3. Combined stress of high altitude and cold. Sixteen rats were placed in a cold chamber (4° C.) at a simulated altitude of 25,000 feet for 4 hours a day, 4 times a week. The rats were sacrificed after 36 to 131 exposures.

4. Combined stress of parabiosis and castration. Fourteen pairs of females were joined by the technique of Brunster and Meyer.[15] Simultaneously a bilateral ovariectomy was performed on one member of the pair. Animals were maintained in this state for 8 to 21 days. Estrogen in large doses constitutes a form of stress which results in hypertrophy of the adrenals.[16] It was for this reason that one of the parabiotic partners was castrated at the outset of parabiosis. The procedure resulted in a marked stimulation of the follicle-stimulating hormone (FSH) in the noncastrated partner, with hypertrophy of the ovaries and the occurrence of a high estrogen level.[17]

All rats were sacrificed by concussion. The hearts were removed promptly, surveyed rapidly under the binocular microscope and fixed in cold formol-calcium. After fixation, the valves were re-examined with the binocular microscope. Gross alterations or vegetations of the valves or chordae tendineae were recorded. Selected areas were taken for histologic study. Lesions were occasionally noted in the fixed valve which were not seen as readily in the fresh tissue. Paraffin sections were stained with hematoxylin and eosin, phosphotungstic acid-hematoxylin (PTAH), the periodic acid-Schiff (PAS) procedure, dilute eosin and methylene blue at various levels of pH, toluidine blue, Alcian blue, and an Alcian blue-PAS-van Gieson combination.

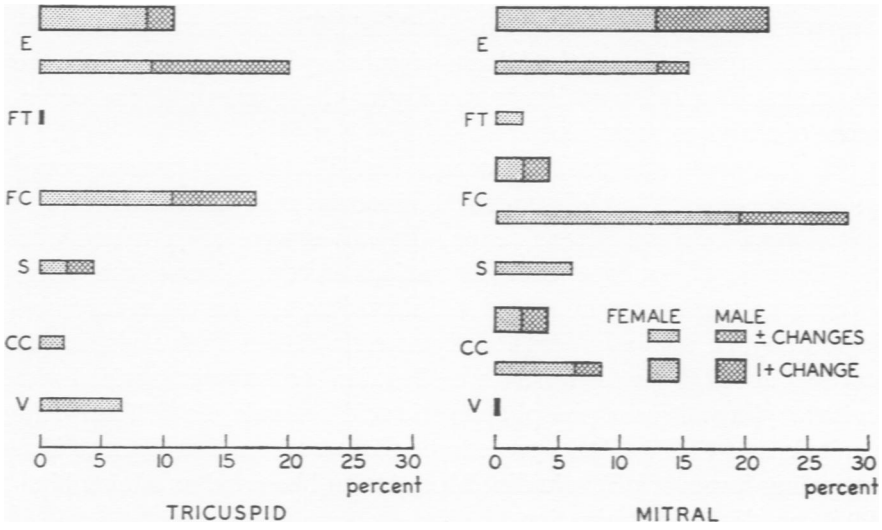
Results

The combined gross and microscopic observations are presented in the form of graphs. The charted alterations represent minimal rather than true values. Serial sections of entire valves were not attempted for practical reasons. Slides prepared from different blocks in the same valve or from contiguous areas deeper in the same block showed considerable variation. It is certain that some lesions which could not be detected in the gross were not sectioned and, therefore, were not included in the final tabulations.

In the valves of control rats kept under the usual conditions of normal care, the changes encountered were focal edema and thickening. No vegetations were encountered. The lesions observed in the special control group kept under ideal conditions are detailed in Text-figure 1. Those showing valvular alterations also exhibited some other abnormality at necropsy (i.e., myocarditis, pneumonia, or nephrosis). In none were these lesions more than moderate (1+); the majority were minimal (±). Vegetations of the fresh platelet variety were found only on the tricuspid valve in two animals, one with nephrosis. Focal edema was the most frequent and most marked change encountered. Occasional valves exhibited focal surface cellularity characterized by hypertrophic and

swollen fibroblasts. Changes in the staining quality of collagen were minimal. Figures 1 to 8 demonstrate the range of alterations encountered in this special control group.

Experimental stresses induced a variety of lesions in and on the valves and chordae (Text-figs. 2 to 5). In order to avoid undue complexity,



TEXT-FIGURE 1. Control group.

All bar graphs are constructed so that the total length of each column represents the incidence percentage of a given valvular lesion in a particular group. The width of each column in turn indicates the degree of severity of the alterations. Minimal lesions (±) are charted only for a special group of normal controls maintained under conditions of minimal stress in order to give the complete range of reaction in these valves. This is also the only chart in which the sexes are indicated separately. There were no variations in the lesions related to the sex of the animals.

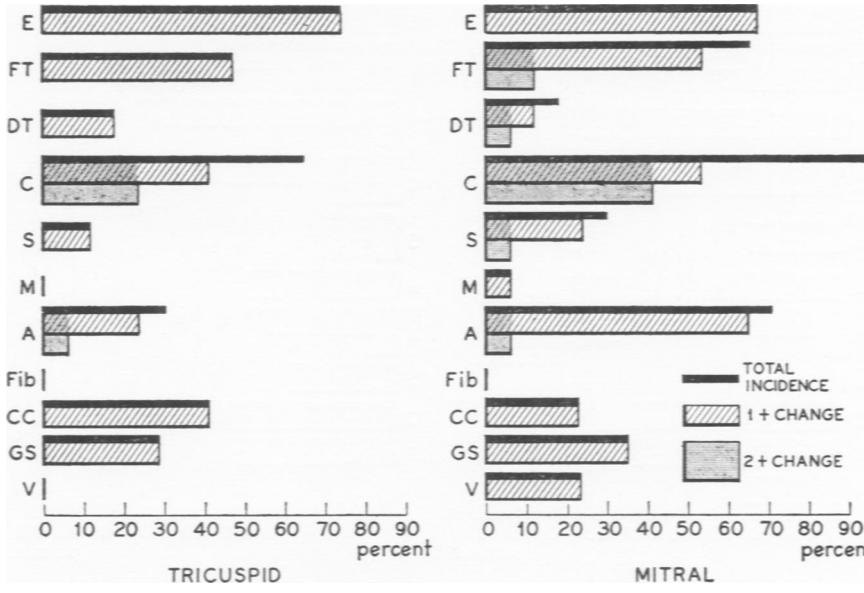
Lesions of moderate and marked severity are designated 1+ and 2+, respectively. The dense black line in the charts pertaining to the experimental groups indicates the overall total incidence of moderate and marked alterations. By overlapping the 2+ and 1+ columns, a graphic representation of the total valve changes is provided.

Key for all graphs:

FE = focal edema
E = diffuse edema
T = thickening
FT = focal thickening
DT = diffuse thickening
C = increased number of cells throughout the valve
S = surface localization
FC = focal clusters of cells
M = monocytic infiltration
A = increased number of Anitschkow cells
Fib = fibroblastic proliferation
CC = collagen changes, including eosinophilia, separation and vacuolation of collagen fibers
GS = swelling of the ground substance
Fd = fibrinoid
V = vegetations

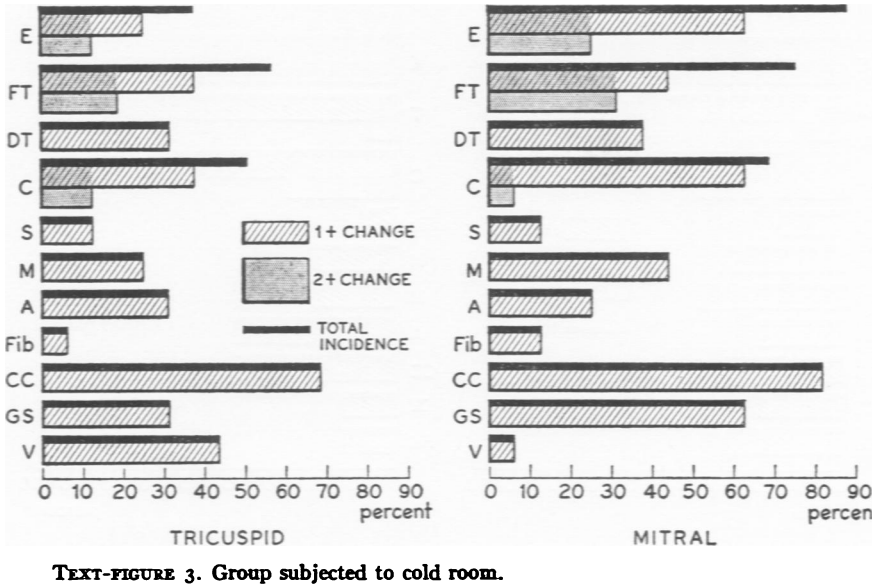
minimal (±) alterations are not indicated. The lesions varied even under seemingly similar conditions.

The lesions in rats subjected to tumbling are given in Text-figure 2. It will be noted that the valves showed edema, focal or linear fibrous

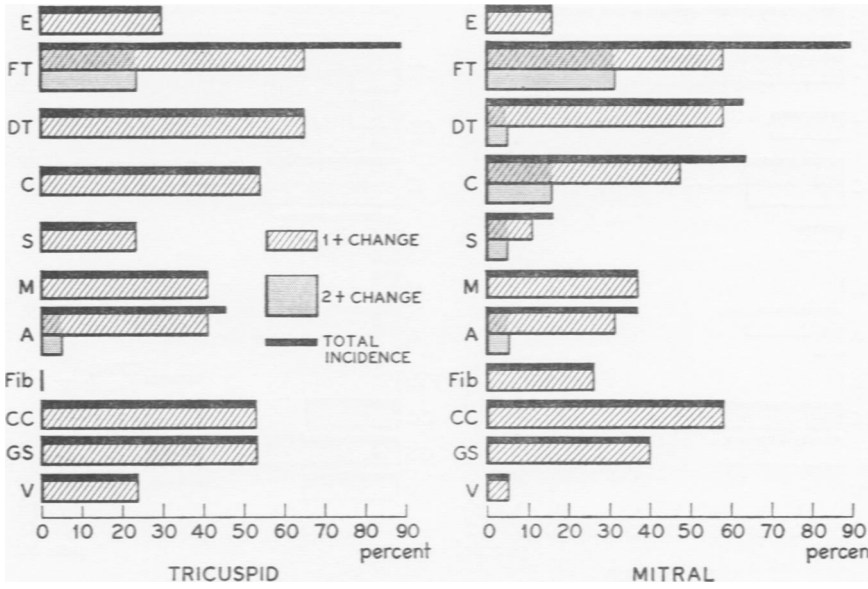


TEXT-FIGURE 2. Group subjected to tumbling.

thickening, increase in cellular content, mainly Anitschkow cells, a few monocytes and mast cells, swelling of the ground substance and increased eosinophilia and spreading of collagen fibers. In general, except for the edema, the alterations were more marked for the mitral valve. Platelet vegetations were limited to this valve (Figs. 9 and 10).

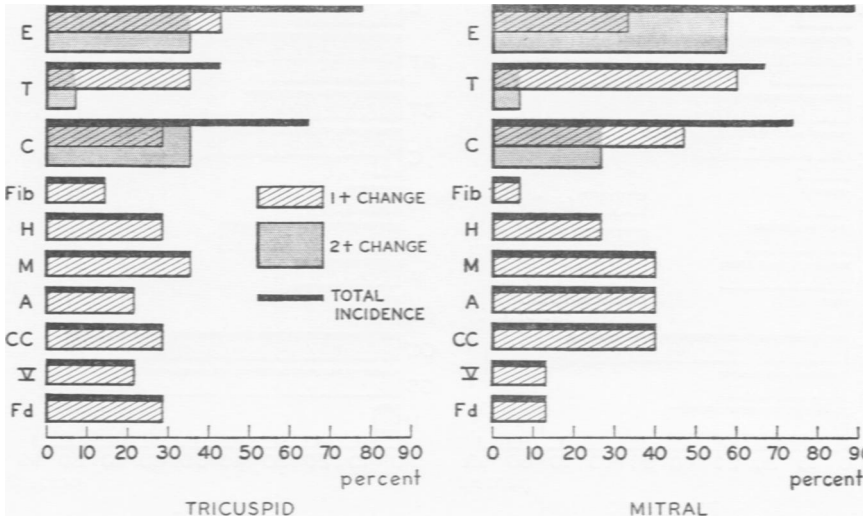


TEXT-FIGURE 3. Group subjected to cold room.



Text-figure 4. Group subjected to high altitude in cold room.

In animals exposed to intermittent cold, the edema, fibrous thickening, increased number of monocytes, Anitschkow cells and fibroblasts, changes in the collagen and the ground substance were more marked (Text-fig. 3 and Figs. 11 and 12). In this instance also the lesions were more severe in the mitral valve. In one case, spontaneous bacterial endocarditis affected both the mitral and tricuspid valves. All other



Text-figure 5. Group subjected to parabiosis with castration of one parabiont.

fresh vegetations were composed, in the main, of platelets and were limited to the tricuspid valve.

The rats exposed to both high altitude and cold exhibited focal and diffuse fibrous valvular thickening (Fig. 14), surface infiltration by monocytes, Anitschkow cells and fibroblasts, and alteration of the connective tissue. The lesions appeared equally in both valves, mitral and tricuspid (Text-fig. 4). Vegetations were also present on both valves in a relatively small percentage of the animals and were of the fresh platelet variety.

The valves in rats subjected to parabiosis and castration of one parabiont showed the most advanced lesions (Text-fig. 5 and Figs. 13, 15 and 16). The mitral valve was more often the seat of marked distortion. In addition to edema, thickening and fibroblastic proliferation, an increase of cellular content, particularly monocytes, and evidence of mitotic activity were very prominent. Changes in the collagen and ground substance were conspicuous, and fibrinoid deposit was observed in both the valve substance and the vegetations.

Special stains for connective tissue were not revealing; the alterations seemed essentially to represent edema and distortion of collagen. The PTAH stain demonstrated remarkably little fibrin in the fibrinoid lesions or vegetations. Alcian blue-staining stroma was increased in staining intensity in most of the valves. In those in which edema was a prominent feature, the intensity was diminished. Extracellular PAS staining was limited to fibers, apparently of collagenous nature. The cytoplasm of some of the larger mononuclear cells, particularly among the castrated parabionts was also PAS-positive. No explanation can be offered for this, although some of the cells probably represented altered mast cells.

The multiple experimental conditions and the small series of rats used in any one group require explanation. Obviously, no statistical evaluation is being attempted. The primary objective was to achieve a technical procedure that would induce early valvular lesions with a degree of consistent uniformity of timing in order to permit their morphologic investigation.

The adrenals in the rats subjected to chronic stress were hypertrophied, and there was a general correlation between the degree of hypertrophy and the severity of the valve changes. An index of hypertrophy was derived by taking the ratio of adrenal weight to body weight of each animal as the numerator, with the ratio derived in a similar fashion for the same size and sex as indicated in the tables of Gray and Addis[18] for normal rats, as the denominator. Hypertrophy was most marked in young rats exposed to intermittent cold and in rats subjected to parabiosis and estrogen stimulation. In the latter group the average of

the indexes (ratio of adrenal weight to body weight) was 1.2659 as compared to 1.0442 in the special control group. In the rats subjected to parabiosis only, the average index of hypertrophy was 1.0592. In those exposed to continuous cold, the average index was 1.1852. For young rats (average weight, 125 to 200 gm.) exposed to intermittent cold, the average index was 1.5182. In older rats (400 to 500 gm.) this index could not be derived because the standard tables of Gray and Addis gave no data for rats weighing more than 419 gm.

Discussion

These studies confirm the observations of others that valvular lesions, including vegetations, can follow different forms of stress in the absence of infection, sensitization or an immunization factor.[1,5-10] The lesions induced suggest an alteration of the valve connective tissue, particularly of its ground substance.

The occurrence of lesions in the valves of normal control animals is significant, and the alterations in the experimental rats must be evaluated with these spontaneous lesions as base lines. The implication is that the valve tissue is quite labile and susceptible to the minor degrees of stress occurring in normal animals. The ready production of valvular lesions by many forms of stress lends further support to such a hypothesis.

The frequent occurrence of fresh vegetations, particularly of the platelet variety, and the low incidence of organized stages of these lesions requires explanation. Apparently the platelet vegetations can undergo rapid dissolution and absorption and do not often show progressive organization. They resemble the platelet vegetations described by Grant, Wood and Jones[19] in the pockets and on roughened surfaces of human valve leaflets. These lesions were thought to favor localization of organisms on the valve, constituting a nidus for bacterial endocarditis. The valvular thickening found in the experimental animals was not ascribable to organization of the platelet thrombi; the thickening appeared to follow the interstitial alterations.

The most common early vegetations were platelet thrombi, which were preceded by changes within the valve proper. The most common alteration in the latter region was characterized by a focal or diffuse edema and an increase in fixed tissue elements. Increased eosinophilia of collagen and intensification of Alcian blue staining were not directly related to the overlying vegetations. Fibrinoid deposit and reactive cellularity were found consistently in relation to the vegetations in the parabiotic group, as were alterations in the staining of the collagen. In only one instance did fibrinoid in the vegetation appear to stem from the under-

lying collagen as suggested by Allen.[20,21] It would appear that vegetations may have more than one mechanism of origin, i.e., adhesion of platelets, fibrin precipitation, or extrusion of altered valve substance.

Some of the variations in the valvular reactions are undoubtedly attributable to differences in the ages of the animals. Although the experimental groups consisted of rats weighing approximately 200 gm. at the outset, the experiments were of variable duration, and the animals were thus of different ages at the time of necropsy. The age factor did appear to have some bearing on the alterations of collagen and thickening of the valves. In the tumbling series the age factor was specifically investigated by dividing the animals into a younger group weighing 200 gm. or less and a more mature group weighing approximately 350 gm. In the control series, the younger rats showed fewer spontaneous alterations, but in the young experimental group there was a heightened susceptibility to the production of lesions.

The varied character and intensity of the lesions in rats subjected to stress under identical conditions cannot be explained. An inherent ability to withstand stress may constitute an individual quality, determined by genetic and endocrine factors or past exposure to stress. The possibility of an endocrine factor is suggested by the hypertrophy of the adrenals encountered in the experimental animals. The well known effect of steroid and pituitary hormones on normal and abnormal connective tissue also favors this possibility.[22,23] It is conceivable that a sensitivity state and systemic infection may act locally on the valvular stroma, at least initially, through the medium of an induced endocrine aberration. A unified concept that the initial alteration in the valve constitutes a common denominator in all vegetations and their transitions as encountered in human disorders of this nature, has been proposed.[11,14,15]

Summary

Heart valve lesions may appear following different forms of stress. The alterations are varied in nature, intensity and frequency. They are characterized by alteration of the valvular ground substance, abnormalities of collagen, the deposition of fibrinoid, fibrous thickening, and an increase in fixed tissue cell content; the latter vary and include Anitschkow cells, fibroblasts, monocytes and mast cells. Orientation in palisade-like manner to the surface is a noticeable feature. Additional features are swelling of the endothelium, and the appearance of platelet or thrombotic vegetations. Only occasionally is there spontaneous bacterial contamination. Similar lesions with considerably less severity appear in normal and pretreated controls.

The significance of these alterations as representing fundamental

preparatory changes to the evolution of nonbacterial and bacterial vegetative valvular disorder as encountered in human disease requires further investigation and thought.

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[Illustrations follow]

Legends for Figures

Except where indicated, photographs are from sections stained with hematoxylin and eosin.

Fig. 1. Five-week-old female control rat (84 gm.). Animal kept under optimal conditions for one week with minimal stress. Mitral valve is thin and transparent. The spherical shadow at its base represents an air bubble trapped beneath the cusp.

Fig. 2. Mitral valve of a 2-month-old female rat (182 gm.) kept under optimal conditions for one month. The fibrosa and spongiosa are normal. × 140.

Fig. 3. Eight-week-old male rat (191 gm.) kept under optimal conditions for 2 weeks. The tricuspid valve is normal throughout except for minimal edema in the chordae tendineae on the left, extending to the papillary muscle.

Fig. 4. A 12-week-old female rat (206 gm.) kept under optimal conditions for 8 weeks. Tricuspid valve has normal structure. × 110.

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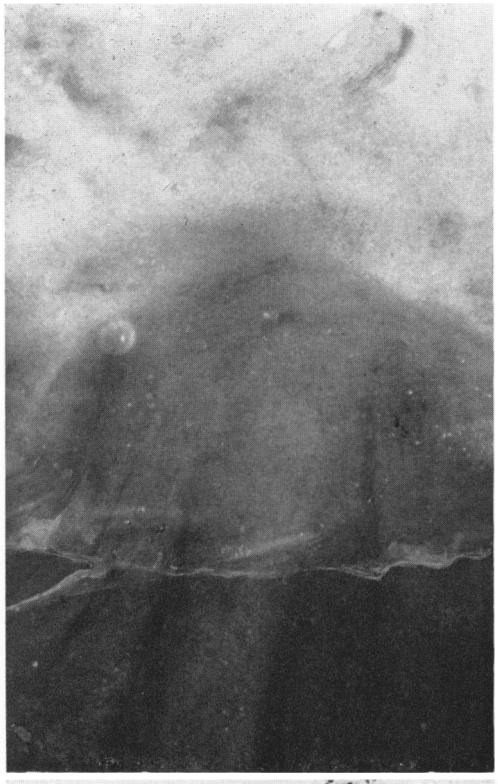

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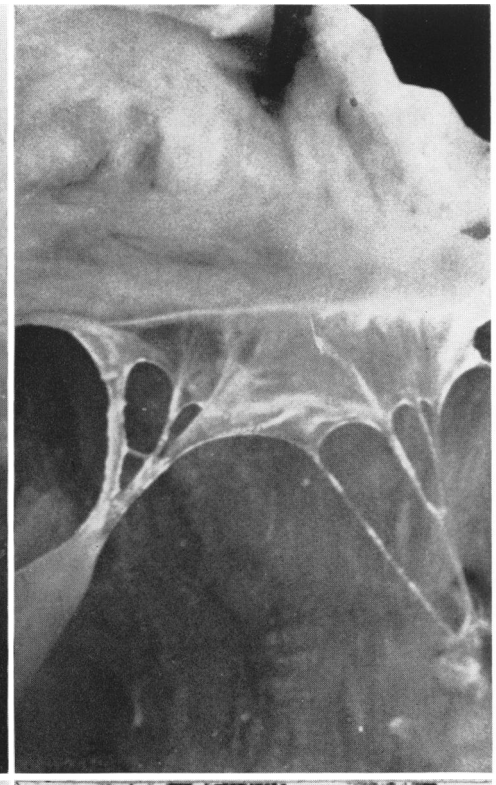

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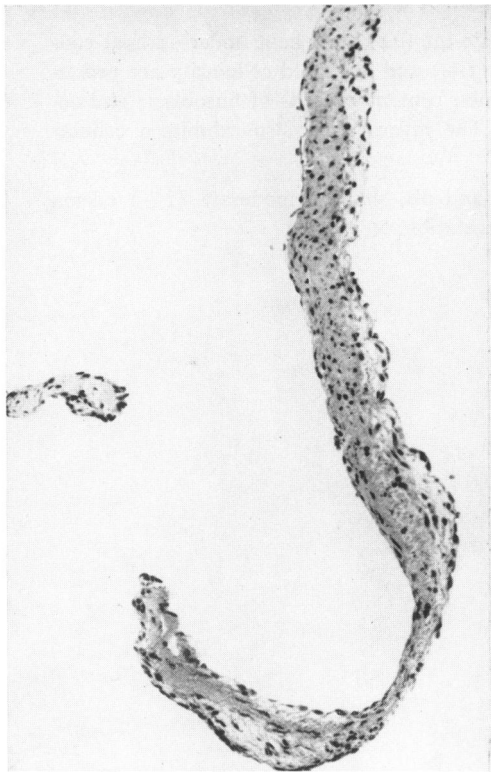

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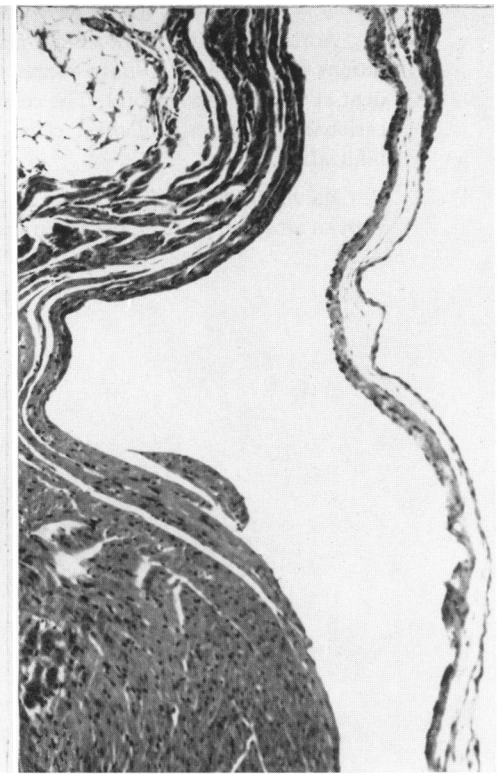

FIG. 5. Section of tricuspid valve in rat whose mitral valve is seen in Figure 1. There is minimal (±) edema in the lower half of the valve. Myocarditis characterized by a diffuse mononuclear infiltration is present in the regional muscle. × 180.

FIG. 6A. Heart valve in a 13-week-old male rat (401 gm.) kept under optimal conditions for 9 weeks. There is edema of the tricuspid valve and its chordae tendineae. A confluent linear platelet and fibrin vegetation is seen on the atrial surface of the valve (lower right arrow), and another large vegetation extends to the left onto some chordae (upper, larger arrow). The kidneys of this rat were large and pale, and the seat of nephrosis with marked dilatation of collecting and convoluted tubules.

FIG. 6B. Valve shown in Figure 6A. A portion of the platelet vegetation at the tip of the valve appears in the lower right. × 90.

FIG. 7. Aortic valve in a 16-week-old male rat (447 gm.) kept under optimal conditions for 12 weeks. Diffuse edema (1+) and increased cellularity are prominent at the tip (upper left). The cellular content consists of fibroblasts and occasional monocytes and mast cells. The myocardium also exhibits a cellular infiltration. × 140.

FIG. 8. Valve illustrated in Figures 6A and 6B, showing moderate (1+) edema with an attached platelet and fibrin vegetation. × 180.

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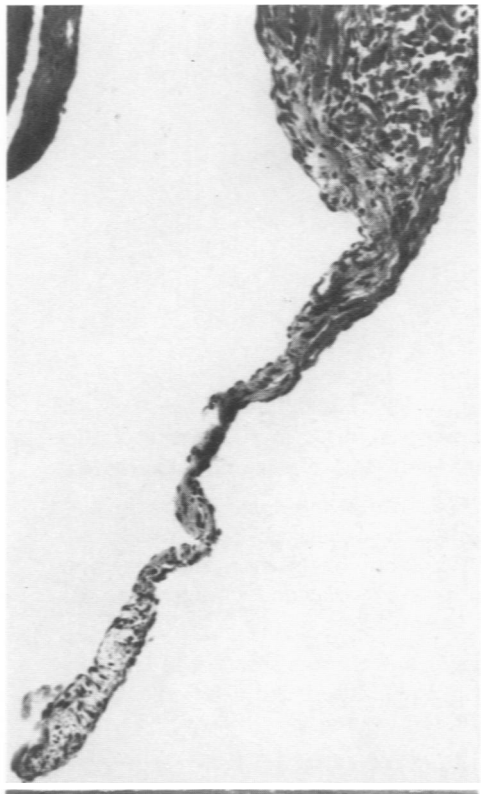

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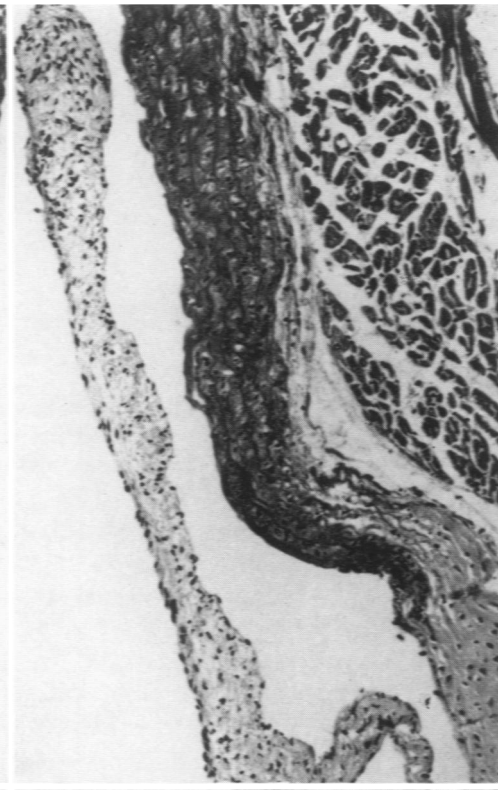

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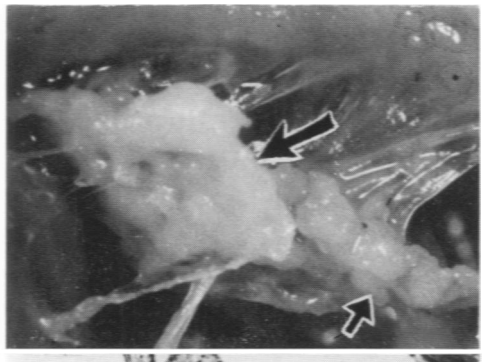

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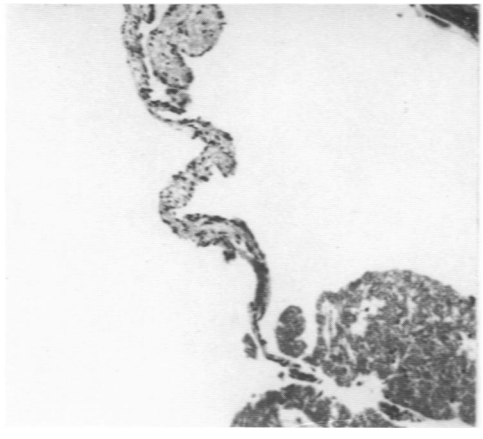

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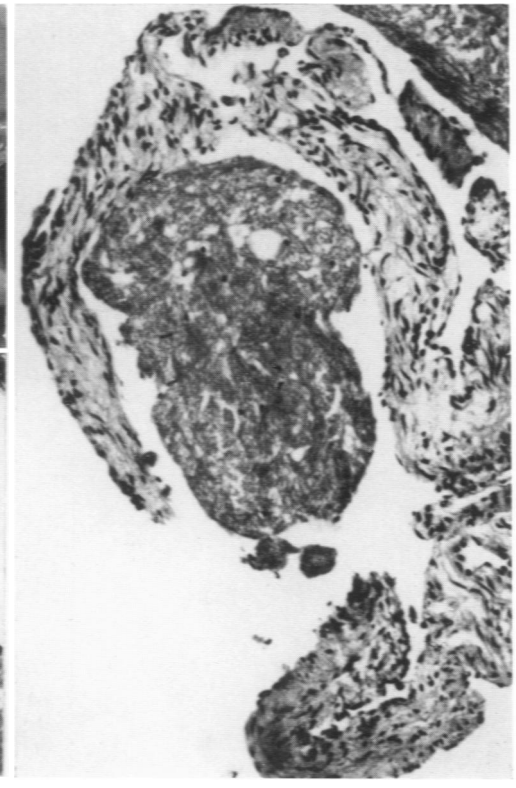


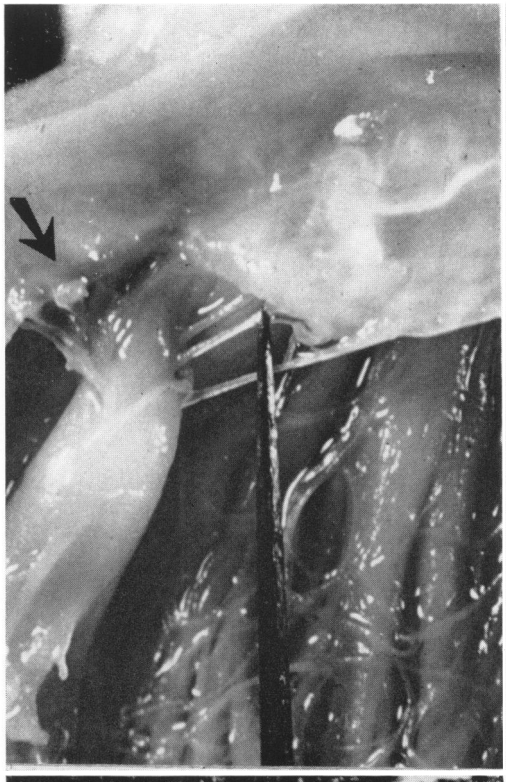
FIG. 9. Rat subjected to 35 tumbling periods. There is diffuse thickening of the mitral valve (at tip of dissecting needle). A small, protuberant vegetation (arrow) is seen on the short cusp and extends a short distance along the chordae.

FIG. 10. Rat subjected to 35 tumbling periods. Aortic valve showing considerable edema (2+), focal eosinophilia and distortion of collagen. Cellularity is composed chiefly of Anitschkow cells, fibroblasts, rare lymphocytes and monocytes. There is surface localization at the tip, focal grouping and a tendency to perpendicular orientation. × 130.

FIG. 11. Rat exposed to cold (4° C.) 82 times. Mitral valve shows adherent blood protein or protruding valve substance (arrow), edema and a few cellular elements. Phosphotungstic acid-hematoxylin stain. ×180.

FIG. 12. A spontaneous bacterial vegetation on the mitral valve. Rat exposed to cold 82 times. The vegetation (black arrow, lower right) contains altered platelets, fibrin and bacteria. At its base at the level of the valve surface, there is an inflammatory exudate, with neutrophils predominating. On the ventricular surface of the valve opposite the bacterial vegetation are 2 small vegetations (hollow arrow) without exudation. These suggest nonbacterial vegetations. PTAH stain. × 150.

9



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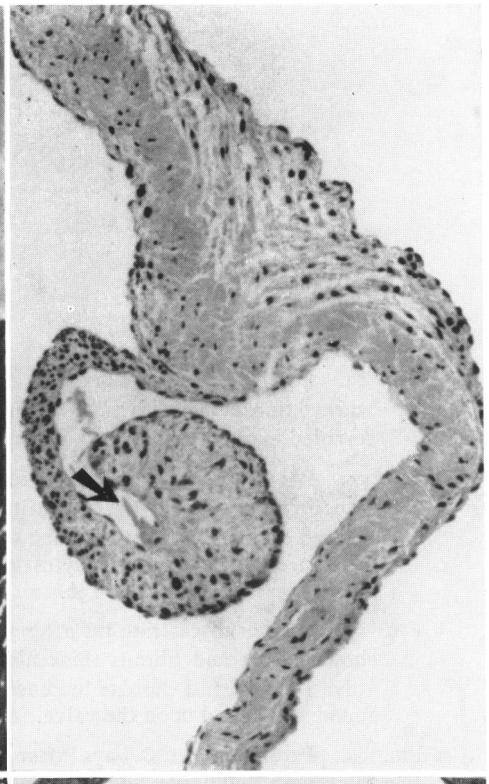

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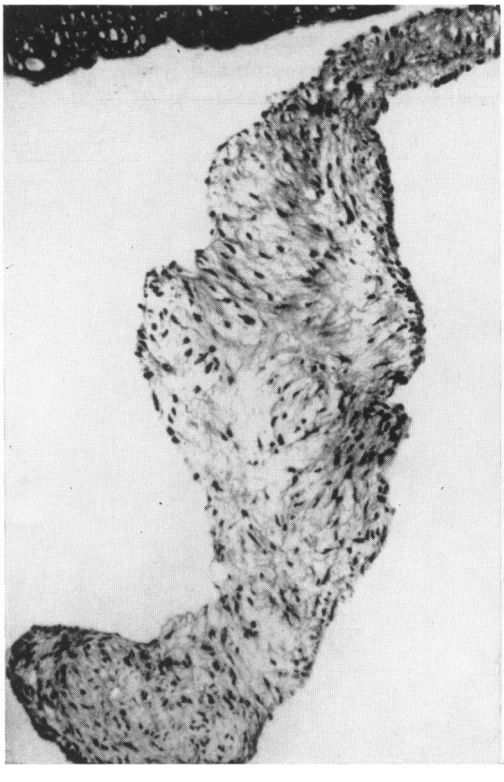

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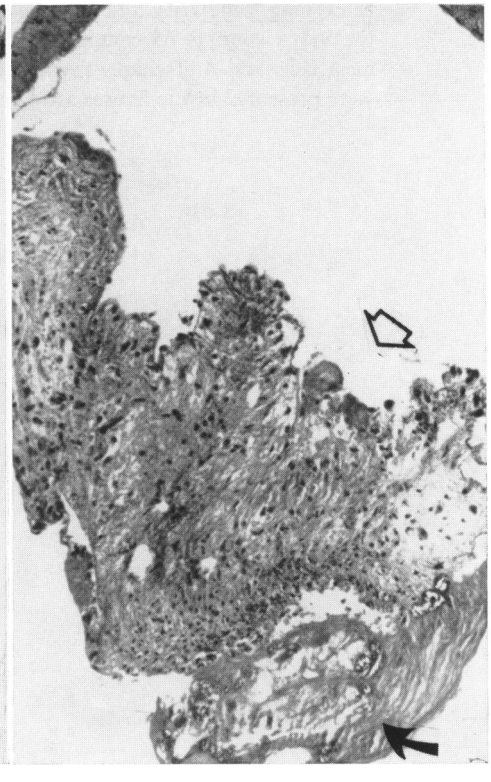


FIG. 13. Parabiotic rat 14 days after operation. Tricuspid valve shows diffuse and marked thickening, with a "billowing sail" appearance. Microscopically this valve revealed marked cellularity and some adherent fibrinoid. See Figure 16.

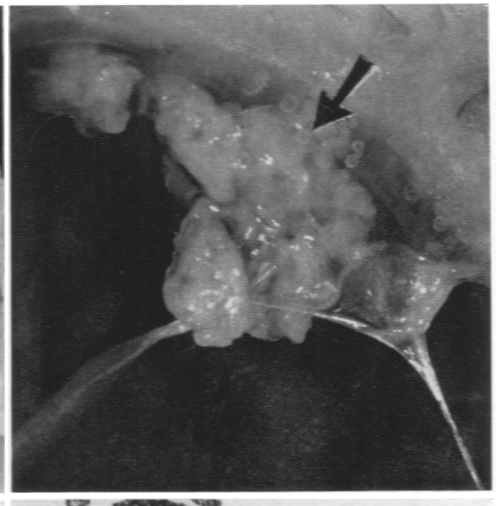
FIG. 14. Rat after 45 exposures to reduced atmospheric pressure (equivalent to 25,000 feet) and cold (4° C.). Mitral valve is thickened and shows edema, increased cellularity with palisading of fibroblasts separated by edematous ground substance. Occasional monocytes and alteration of collagen are apparent. No vegetation is manifest. × 130.

FIG. 15. Partner of castrate rat after 10 days of parabiotic union. Tricuspid valve shows edema and fibrous thickening. Nonbacterial vegetations appear on the valve (arrow) and chordae tendineae above the papillary muscle. Fibrinoid was found within and upon the valve.

FIG. 16. Parabiotic rat 8 days after operation. Tricuspid valve shows marked thickening, cellularity and adherent fibrinoid (arrow). The cellular infiltrate in the valve consists of compactly placed monocytes, hypertrophied fibroblasts, mast cells and Anitschkow myocytes. Only a few neutrophils and lymphocytes were present. Mitotic figures are frequent in the cellular infiltrate. × 90.

13

15



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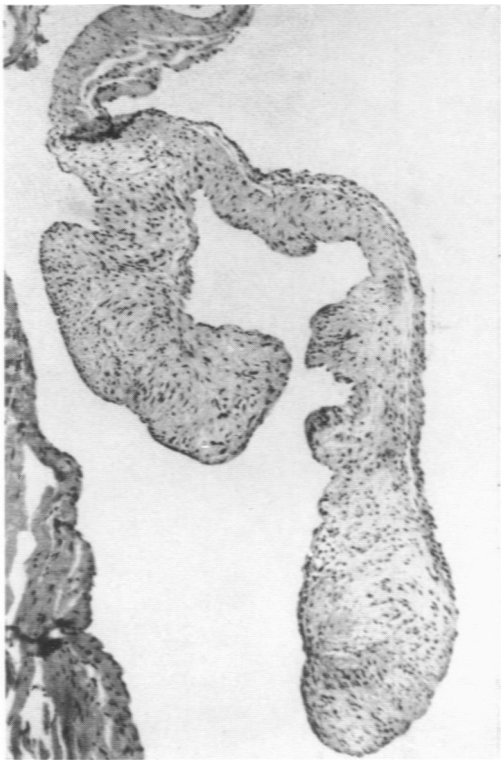

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