CARDIAC VALVE AND AORTIC LESIONS IN BETA-AMINOPROPIONITRILE FED RATS WITH AND WITHOUT HIGH SALT

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Beta-aminopropionitrile (BAPN) interferes with the formation of firmly aggregated collagen molecules, though the production of the molecular amino acid chain of pro-collagen itself is not impaired.[1-7] It has been suggested that the effect of lathyrogenic agents on collagen was indirect and was mediated by an altered mucopolysaccharide metabolism.[8] A block in the relation of chondroitin sulphates A and C with protein,[9] or a possible destruction of the chondroitin sulphate of the connective tissue ground substance[10,11] have also been suggested. The cardiac valves contain a considerable amount of ground substance and show edematous swelling and other changes following general stress.[12]

The purpose of this study was to determine if BAPN had an effect on cardiac valve tissue. High salt diet, which usually yields hypertension, and subsequent alteration of electrolytes in the walls of arteries[13] in the rat, combined with BAPN, known to yield aortic lesions in this animal, appeared to constitute a promising model to determine such effects, additive or independent, on the heart valve and the aorta. The enzymatic reactions studied helped delineate the early changes.

Material and Methods

Seventy-two weanling Sprague-Dawley female rats were divided into 3 groups: 24 rats were fed with 0.2 per cent BAPN in Purina® chow (BAPN diet contained 0.9 gm BAPN per pound Purina® chow in pellet form), 36 rats were fed with 0.2 per cent BAPN in high salt diet (BAPN diet plus high salt contained 0.9 gm BAPN per pound Purina® chow which had in addition 11 per cent NaCl by weight; these diets were prepared in pellet form by General Biochemicals, Chagrin Falls, Ohio) and 12 rats were fed with regular Purina® chow for controls. Six rats were discarded because of postmortem changes (Table I).

The diets were started on the 25th day of life; the duration of the experiment was from 17 days to 48 days. One rat was killed after 60 days of the high salt diet with BAPN. The rats were sacrificed periodically and dead rats without postmortem changes were also subjected to gross and histologic study. The hearts and the aortas

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were examined with a binocular dissecting microscope. Paraffin sections were prepared and stained with hematoxylin and eosin, elastica van Gieson, phosphotungstic acid hematoxylin (PTAH) and a combination of periodic acid-Schiff(PAS)-alcian blue picric acid methods.

The heart valve and the ascending aorta in 12 rats, 4 rats each from control, BAPN and BAPN in high salt diet groups (with experimental durations of 18, 26, 37

TABLE I

EXPERIMENTAL CONDITIONS AND TIME OF SACRIFICE

Exper. cond.	Total rats	No. rats studied	Time of sacrifice (days)				
			17–24	25–32	33–40	41–48	60
BAPN alone	24	21	5 (2)	8 (3)	5 (5)	3 (1)	0
BAPN with high salt	36	33	9 (3)	11 (6)	8 (4)	4 (3)	1 (0)
Control	12	12	3	3	3	3	

Parenthesis is number of rats with severe heart valve lesions.
No rat developed severe heart valve lesions in the control group.

and 44 days) had histo-enzymatic studies done. Cryostat sections were prepared from unfixed fresh tissues. These were fixed in Baker's cold formalin for 30 minutes prior to enzymatic reactions. The enzymes studied were: 1) reduced diphosphopyridine nucleotide diaphorase (DPNH-diaphorase), 2) adenosinemonophosphatase (AMPase), 3) adenosinetriphosphatase (ATPase), 4) alkaline and 5) acid phosphatase reactions. The methods used were: the DPNH-diaphorase reaction after Novikoff,[14] using Nitro-BT tetrazolium; the ATPase and AMPase reactions after Wachstein-Meisel; [15] and the acid and alkaline phosphatases after Gomori.[16,17]

For the autoradiographic studies, tritiated L-proline (L-proline-^{3}H) was injected intraperitoneally into 3 rats in each group, i.e., the intact control, BAPN, and BAPN in high salt diet groups; a total of 9 rats were thus used. One μc of L-proline-^{3}H per gm body weight was administered to 1 rat in each of the 3 groups mentioned on the 26th day and to 2 rats each in the corresponding groups on the 32nd day of the experiment. The former rats (26th day of the experiment) were sacrificed 8 days later and the latter (32nd day of experiment) were sacrificed 48 and 108 hours later. Paraffin sections of the heart valve and the ascending aorta for autoradiography were deparaffinized and rinsed with running water for 4 hours. Three test slides for each group and 2 sets of final sections were prepared. The test slides were used to determine the optimal exposure period. One set of slides was dried in air after washing with water and was not coated with colloidin. The other set was coated with 0.2 per cent of colloidin twice to prevent diffusion and unevenness of the surface. NTB-3 (Eastman Kodak Co., Rochester, N.Y.) was diluted with distilled water (3 to 1) and the 2 sets of slides were coated and dried in usual manner. The exposure periods as determined by the test slides were 13 weeks for the dosage of 1 μc L-proline-^{3}H. Microphotographs were taken of the equivalent area of the control and experimental rats and a count of the silver grains was made on identical (8 × 10 in.) enlargements through a sq cm overlay. The number of silver grains per sq cm was counted on the enlargements with times 1,420 magnification from the original sections. The ratio of the counts for the BAPN treated rat to the normal was determined.

For the quantitative determination of radioactivity in the heart valves and aorta, adventitial tissues of aorta were stripped and blood was removed by filter paper. These samples were weighed on Schoeniger papers, dried in a 60° C oven overnight and underwent combustion in oxygen in Schoeninger flasks. The water was trapped by placing the Schoeniger flask in a dry-ice acetone bath. Five cc of absolute alcohol was added and 3 cc was taken for counting. Scintillation fluid was added and the vials counted in a Packard Tri-Carb. Under the conditions of counting, 1 μc tritiated water was equivalent of 345×10^3 counts per minute.

RESULTS

Heart Valve Lesions. The heart valve lesions were classified as — or ±, 2+ and 3+ lesions according to the degree of edema, cellularity, collagen changes and fibrous thickening. The — or ± lesion, with very limited edema and minimal cell reaction (Figs. 1A and 1B) was considered normal. Eleven of 12 intact control rats had normal valves by this definition. The 1+ lesions represented a minimal alteration with some edema and cell increase (Figs. 2A and 2B). The 2+ and 3+ lesions were considered severe lesions. Grossly a nodular or a diffuse opaque thickening of the valve was present in valves altered in this manner. Microscopically the lesions showed marked alcian-blue-positive edema, with an obvious cellular reaction (Figs. 3B and 4B). The valve was often increased 4- or 5-fold in thickness compared to the valves in control rats, and a cellular reaction was prominent (Fig. 4B); the reactive cells were large and tended to aggregate near the surface of the valve.

Eleven of 21 rats (52 per cent) in the BAPN diet group and 16 of 33 rats (48 per cent) receiving BAPN and high salt, showed severe valvular lesions. There was no significant difference in the incidence of severe heart valve lesions in these two groups.

Valvular Enzymatic Reactions. Normally there was a rich AMPase content in the young rat when the cell reaction and edema were present (Fig. 5A) but this was relatively limited in the normal valve in adult rats (Fig. 5B). (Figure 6 is the hematoxylin and eosin stained section corresponding to Figures 7, 8 and 9.) In the distorted valve the AMPase reaction was strongly positive and was increased in the stroma (valve proper) as well as in the endothelium (Fig. 7), while the ATPase reaction was positive in the surface endothelium and negative or only minimal in the stroma (Fig. 8). The alkaline phosphatase reaction product was seen in the endothelium and in the fibrous tissue of the stroma, but not in most of the reacting cells (Fig. 9). No difference was found in the enzymatic reactions in the heart valves in the groups with BAPN diet and those with BAPN and high salt diet. In the reacting cells in both groups the DPNH-diaphorase reaction showed a heavier formazan precipitation and acid phosphatase positive granules were variable in size.

Dissecting Aneurysms of the Aorta with and without Rupture. Dissecting aneurysms or aortic ruptures were most frequently encountered at the arch and in the ascending segment. The histologic findings were the same as that given by other authors[18-22]; the dissecting aneurysms were located mainly in the outer one-third of media. Active fibroblastic proliferation followed dissection. Granulation tissue involved the adventitia and seemed to delay further dissection and rupture.

There was no qualitative difference in the histologic findings in the groups receiving combined high salt and BAPN diet and BAPN diet alone. Dissecting aneurysm or rupture, however, were encountered sooner and more frequently in the group fed BAPN combined with the high salt diet. The first rupture developed on 17th day in this group and aortic rupture occurred in 19 of 32 rats (59 per cent) during the 7 weeks of the experiment. One rat was sacrificed on 60th day; there was no dissecting aneurysm. In contrast in the rats on BAPN diet without high salt, the first rupture or dissecting aneurysm developed on the 26th day; this occurred in 6 of 21 rats (29 per cent) during the 7 weeks of experimental period (Table II).

TABLE II

RELATION OF AORTIC AND SEVERE HEART VALVE LESIONS

Exper. cond.	No. of rats	With severe valve lesions	With dissection or rupture of aorta: No. of rats	1st Appearance	Heart valve: With severe lesions	Heart valve: Without severe lesions	Severe valve lesions without rupture
BAPN *	21	11 (52%)	6 (29%)	26 days	3	3	8
BAPN + salt †	32 ‡	16 (50%)	19 (59%)	17 days	12	7	4
Control §	12	0	0				

* BAPN = 0.2 per cent of beta-aminopropionitrile in Purina® chow.
† Salt = 11 per cent NaCl.
‡ No. of rats–48 days of experimental duration.
§ Control-fed with regular Purina® chow.

Enzymatic Reactions of Aorta. The enzymatic reactions were studied only when the aorta showed distortion (thickening or dilatation) but was not ruptured. The thickened region was compared with a normal segment in the same cross section of the aorta as well in the control rats. An increase in reaction was noted with the AMPase (Figs. 10A and 10B) and the ATPase procedures (Figs. 11A and 11B) between the elastic fibers in the distorted thickened region of aorta. The DPNH-diaphorase reaction (Figs. 12A and 12B) was also increased in cells of the altered area and seemed to be related to a distortion of aortic elastica. The alkaline (Figs. 13A and 13B) and acid phosphatase reactions showed no significant change before dissection and rupture, except that the altered elastica was defined by a precipitate along the refractile fibers. Though the elastic fibers showed no positive reaction, the focal alterations of elastic fibers were made more apparent by these enzymatic reactions (Figs. 10 to 13). In the distorted aorta, not yet ruptured or dissected, the elastica exhibited separation, swelling and there was a loss of continuity of the refractive lamellae.

Autoradiographic Studies. The autoradiographic studies revealed a difference in the uptake between the control rats and the rats on BAPN diets, but no significant difference in the BAPN diet groups with or without high salt. This was particularly prominent in the rats sacrificed 108 hours after administration of the tritiated L-proline (Table III).

TABLE III

COMPARISON OF AUTORADIOGRAPH GRAIN COUNTS * IN CONTROL AND BAPN FED RATS WITH HIGH SALT DIET

	Control	BAPN & high salt	Control/ BAPN & high salt
48 hours			
Heart valve	0.506	0.810	1.00/1.60
Aorta	0.847	1.763	1.00/2.13
108 hours			
Heart valve	0.275	1.018	1.00/3.70
Aorta	0.666	2.424	1.00/3.64

* No. of grains per sq cm with a magnification of × 1,420 from the original slides.

The BAPN diet rats showed more of an uptake at this time in the heart valve (Figs. 14 and 15); the density of reduced grains was 0.275 per sq cm for the control and 1.018 for BAPN diet rats (both enlarged times 1,420 for counting grains), the ratio of control to BAPN with high salt diet rat was 1.00 to 3.70. Actual counts in the scintillation well counter of radioactivity in the valve tissue, however, revealed a ratio of 1.00 to 1.89 in comparing the control to the rat on BAPN plus high salt. The uptake was also more prominent in the dissected aorta in rats on BAPN diet with high salt. The density of the silver grains was 0.66 for the control as compared to 2.42 per sq cm in the rats on BAPN diet with high salt; the ratio was 1.00 to 3.64 (Figs. 16 and 17). Actual instrumental counts upon a part of the aorta without dissection was also higher in the rat on BAPN plus high salt than in the control rat, the ratio being 1.00 to 1.92. Yet the rats sacrificed 48 hours or 8 days later showed less difference between the control and the animals on BAPN with high salt. The control aorta exhibited a grain count of 0.85 per sq cm. The count for the aorta in the rat on BAPN with high salt was 1.76 per sq cm; the ratio being 1.00 to 2.13. In the heart valves of rats sacrificed 48 hours after L-proline-^{3}H administration, the uptake in those on BAPN with high salt diet was somewhat more than in the controls (Figs. 18 and 19). The density of reduced silver grains was

0.51 for the control and 0.81 per sq cm for rats on BAPN with high salt diet; the ratio being 1.0 to 1.6. An actual instrumental count of the valve tissue in the rats on the BAPN diet with high salt was higher than the control rats, with a ratio of 1.0 to 1.69.

The above data was inadequate for statistical analysis; it has been included to indicate trends only and to note that the actual uptake counts did parallel the grain counts in the autoradiographs. Additional uptake studies were done. A single dose of 4 μc per gm body weight of tritiated L-proline was injected intraperitoneally on the 18th day of the experiment in 12 rats in each of the 3 groups, a total of 36 rats: a) intact controls, b) rats on BAPN diet only, c) rats on BAPN plus high salt diet. Four rats were sacrificed in each group at 24 hours and 2 in each group after 72 hours. Four rats on the BAPN diet with high salt died and were discarded. Rats sacrificed after 96 hours included only 2 rats in the group on BAPN with high salt. Because only a minute amount of heart valve tissue could be collected from each rat, to obtain actual counts with the scintillation counter, heart valve and aortic tissue from each of 2 rats in the same group were pooled. The counts on the tissues from the rats on BAPN diet alone and those on BAPN with high salt were compared with the controls throughout (Table IV). The data

TABLE IV

RATIO OF RADIOACTIVITY COUNTS

Exper. cond.	24 hours		72 hours		96 hours	
	Valve	Aorta	Valve	Aorta	Valve	Aorta
Control	1.000	1.000	1.000	1.000	1.000	1.000
BAPN alone	2.912	1.216	2.928	1.453	2.005	2.125
	3.382	1.251			1.881	1.746
					1.887	1.902
Mean	3.147	1.233			1.924	1.924
BAPN with high salt	3.672	1.296	3.370	1.085	1.837	1.950
	3.432	1.046				
Mean	3.552	1.171				

Note: A single dose of 4 μc per gm body weght tritiated L-proline given intraperitoneally.

seemed to indicate a relative increase in uptake of tritiated L-proline in both groups of rats on BAPN.

Comment. In this experiment there was no significant difference found in the incidence of severe heart valve lesions in the groups of rats fed BAPN with or without high salt diet. Severe heart valve lesions were encountered frequently in this experiment; in 52 per cent of rats on BAPN diet alone and in 49 per cent of those on BAPN combined with high salt diet. The high salt diet seemed to contribute little to the development of valve lesions; the valve lesions were probably more directly related to the administration of BAPN. High salt diet alone in another experiment,[23] failed to yield a high incidence of severe heart valve lesions (less than 10 per cent) in rats with hypertension; in this latter experiment 66 per cent of rats on an identical experimental diet but without BAPN developed moderate hypertension (over 180 mm Hg) in 4 weeks of a high salt diet. Blood pressures were not taken in the present experiment because the rats were too small for the instrumentation available.

A higher rate of dissecting aneurysm and rupture was encountered in the rats fed BAPN combined with high salt. This suggested that the high salt diet was significant in the development of aortic dissection. The alterations of electrolytes in the aortic wall induced by a high salt has been noted[13] and may represent a contributory factor in the high incidence of aortic lesions in rats on a high salt diet combined with the BAPN.

A definite relationship of dissecting aneurysm to severe heart valve lesions was not found. In the group of rats on the BAPN diet alone, 3 of 6 rats with dissecting aneurysms had severe heart valve lesions; 8 rats with severe heart lesions did not have aneurysm. A high coincidence existed, however, between the severe heart valve lesions and dissecting aneurysms or aortic ruptures in the group given BAPN combined with high salt diet; 12 rats had severe valve lesions in 19 rats with dissections. Though the valve lesions could have been attributed to the BAPN, they might as well have been due to the stress effect of the dissecting aneurysms and aortic ruptures (Table II).

A block in collagen maturation was found between the tenth and 15th day in carrageenin granuloma experiments carried out by Hurley and Ham.[7] Heart valve lesions were encountered in the early stages and throughout the entire experimental period of this study. It may be assumed that BAPN intoxication affects active collagen formation in young growing rats. Some interference with valvular collagen maturation might be expected with an increase in the ground substance, as has been suggested.[1–7] An increase of the alkaline phosphatase reaction in the valve proper indicated collagen formation as demonstrated by Danielli[24] in the healing wound.[25,26] The reacting cells in the heart valve showed formazan precipitation with the DPNH-diaphorase reaction, and an increase in acid phosphatase positive granules seemed to be associated with collagen synthesis. Reactive fibroblasts and muscle cells, associated with dissecting aneurysms, are associated with active collagen synthesis.[27] Recent data by Orbison, McCrary and Callahan[28] and other investigators [1–3] indicate an increase in soluble collagen and an increased hydroxyproline excretion in lathyritic rats; this may take origin from young reactive cell areas. There is an increase in soluble collagen in the lathyritic animal.[1,3,4] Active collagen synthesis was also indicated by the autoradiographic studies. The radioactivity or uptake of L-proline-^3H was significantly increased in the BAPN diet rats in both the heart valves and the aorta, when compared to controls. The increased uptake of the tritiated compound was correlated with cell reaction and the presence of increased ground substance. Rats sacrificed 108 hours after administration of the compound showed the most obvious difference in the uptake compared to the intact animals. More precise data with statistical evaluation should be obtained, however, by a further study of radioactivity and uptake of L-proline-^3H in a larger series.

Concerning the alteration of elastic fibers in the aorta, Bean, Baird and Ponseti,[10,11] and Grant, Hathorn and Gillman [29] stated that the elastica did not appear abnormal at first, while Walker and Wirtschafter,[21,22] Menzies and Mills [30] and Churchill, Gelfant, Lalich and Angevine [31] claimed that lytic changes were discernible. The enzymatic reactions in the present study revealed changes in elastic fibers in the early stages of aortic alteration. Keech [27] demonstrated aortic alteration by electron microscopy and confirmed an increase in muscle cells. An increase of AMPase, ATPase and DPNH-diaphorase in the altered area may be attributed to the proliferation of muscle cells, and possibly some alteration in them. An increase of the AMPase reaction was found in the mesenteric and myocardial arteries in the normal appearing segment of vessels in rats with high salt diet induced arteritis.[32] Though the significance of the enzymatic findings is not clear, the relation of altered elastic fibers and an increase of enzymatic reaction is of interest.

The increase in solubility of young collagen in saline may favor weakening of the aortic wall and thus promote aneurysm and rupture. A synergistic action of such enhanced solubility should be considered. This is particularly the case in view of the increased concentration of sodium in the aorta demonstrated by Koletsky,[13] increased salinity favoring solubility. This implies alteration of connective tissue metabolism, the possibility of enhanced solubility and the loss of some products or precursors, with a secondary loss of tensile strength followed by dissection and rupture.

Conclusion

Sixty-six Sprague-Dawley female weanling rats were divided into 3 groups; 1 was given BAPN (0.2 per cent), another BAPN in high salt diet (0.2 per cent BAPN, 11 per cent NaCl in Purina® chow), and the third a regular diet of Purina® chow alone.

A high incidence (over 50 per cent) of severe valve lesions developed in rats on BAPN. There was no difference in the production of the valvular lesions of this severity in the BAPN groups with and without high salt. There was no direct correlation between the presence of aortic aneurysm and the severe heart valve lesions. BAPN in the diet was, however, correlated with severe heart valve lesions.

In altered valves, the AMPase reaction was increased in rats on BAPN. There was an apparent increase in acid mucopolysaccharide, as judged by the alcian blue positivity.

An increase in the uptake of tritiated L-proline in the heart valve was found. An increased uptake was also found in rats on BAPN, particularly in the regions of dissecting aneurysms. An increase in the alkaline phosphatase reaction also suggested new collagen formation at these sites.

The alterations of elastica in the distorted aorta were more easily observed by enzymatic reactions in sites of dissection and aneurysm, without, however, any specific reaction products in sites of elastic tissue.

The high salt diet combined with BAPN produced a higher incidence of aortic dissecting aneurysm and rupture and these occurred sooner than in the group given the BAPN without high salt.

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[*Illustrations follow*]

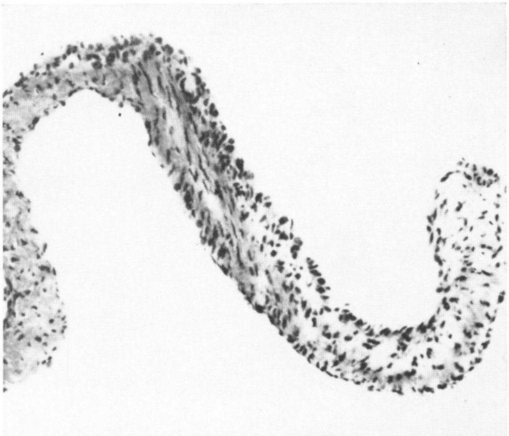
Legends for Figures

Fig. 1A, mitral valve; Fig. 1B, tricuspid valve. Rat on the BAPN diet without high salt for 26 days. Both valves show minimal edema, graded (—). Hematoxylin and eosin (H&E) stain. × 100.

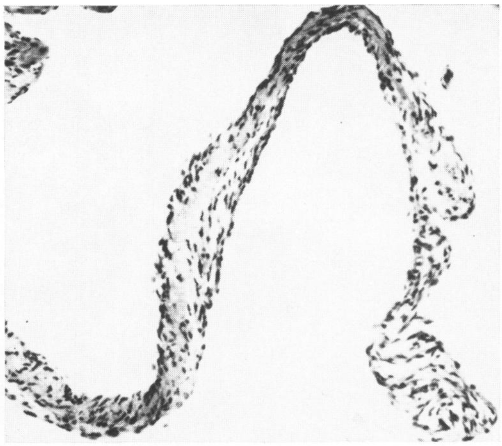
Fig. 2. Tricuspid valve, rat fed BAPN without high salt for 44 days.
A. Macroscopically, there is minimal edema at edge of the valve (arrow).
B. Microscopically there is moderate edema with minimal cell reaction, graded 1+. H&E stain. × 100.

Fig. 3. Mitral valve, rat fed BAPN without high salt for 34 days.
A. The mitral valve shows opaque thickening of the anterior and posterior cusps (arrows).
B. The anterior cusp shows edema, thickening and considerable cell reaction, graded 2+. H&E stain. × 90.

1A

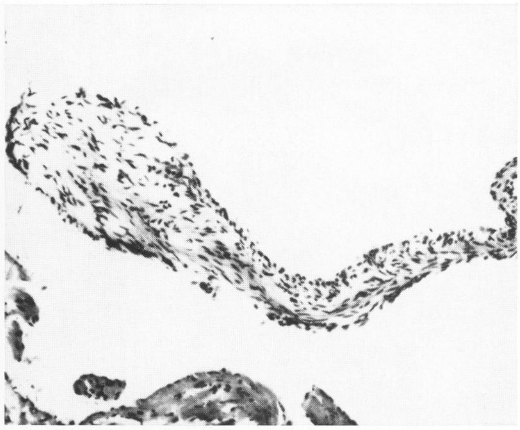


1B



2A

2B



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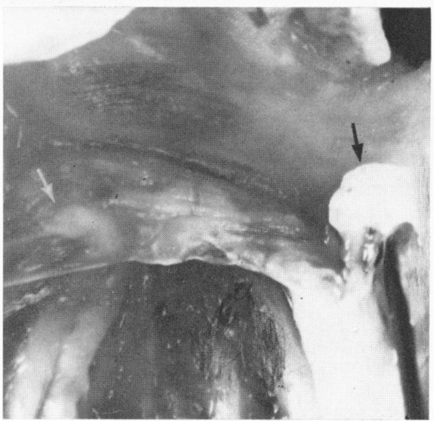

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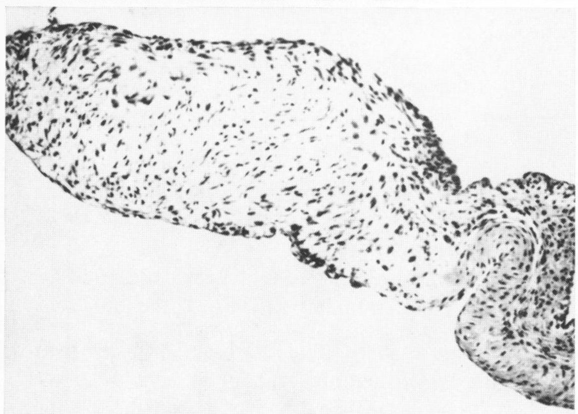
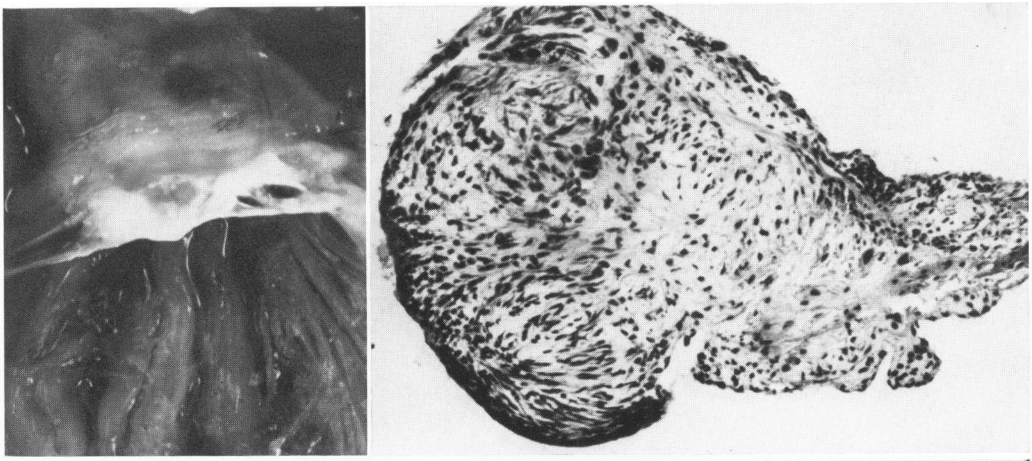


4A 4B



5A 5B

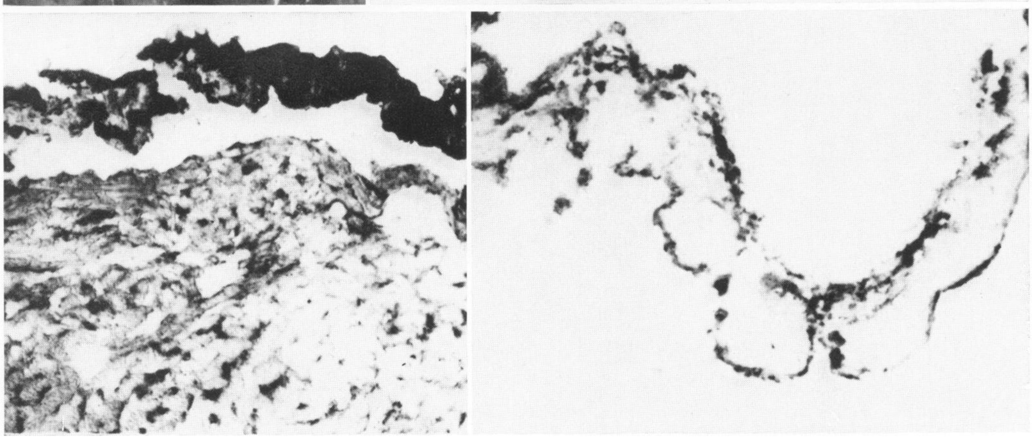


FIG. 4. Mitral valve, rat fed BAPN without high salt for 45 days.
A. Opaque thickening is present toward the edge of valve and chordae tendineae.
B. The anterior cusp exhibits marked edema and cell reaction, graded 3+. H&E stain. × 100.

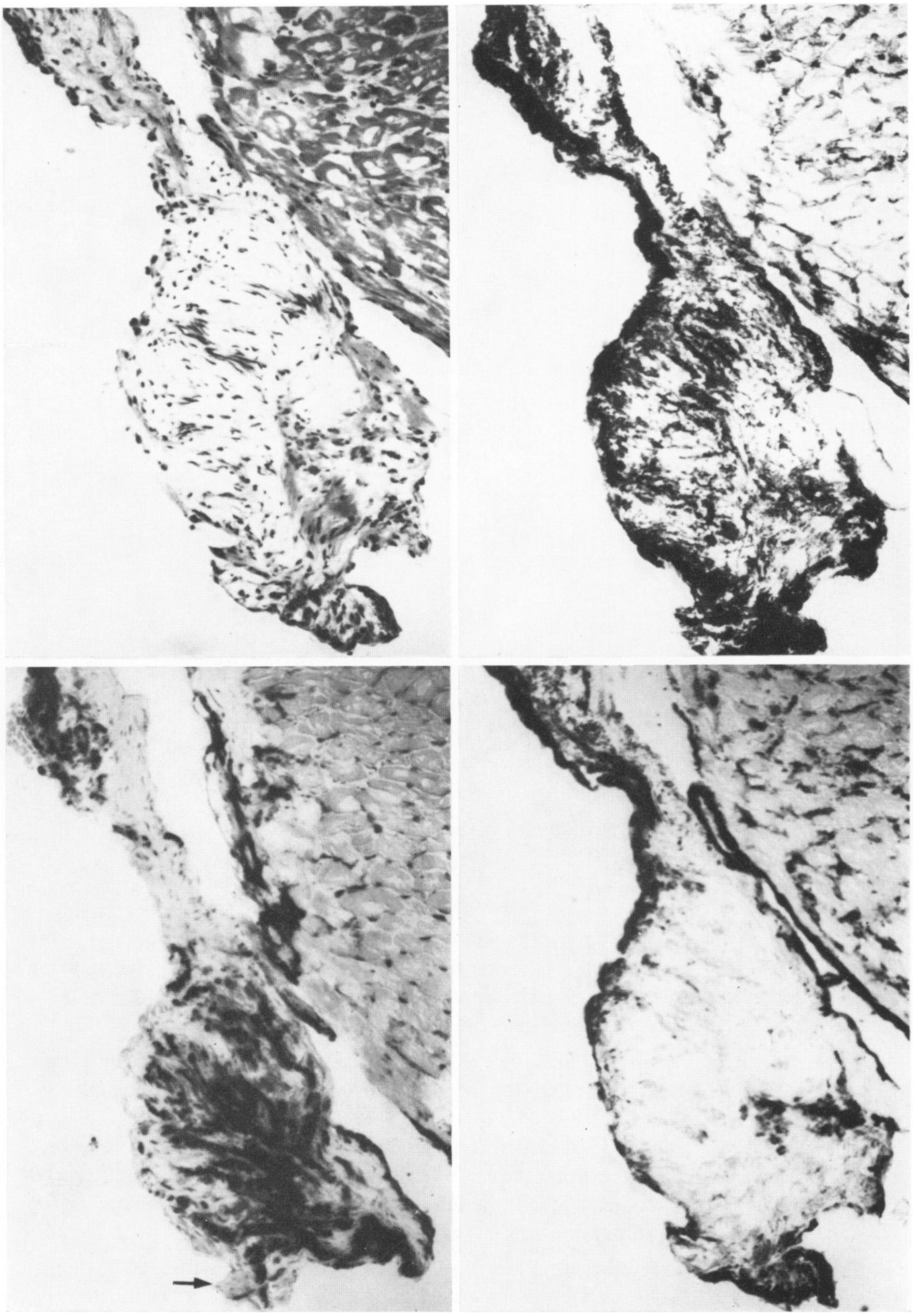
FIG. 5. AMPase reaction in the tricuspid valve, untreated control rat.
A. Young rat, 30 days of age. The AMPase reaction is prominent in the valve stroma. × 95.
B. An untreated intact rat, 80 days of age. The normal valve shows the AMPase reaction mainly in the endothelium. × 95.

Figures 6 to 9 inclusive are cryostat sections of a mitral valve from a rat fed BAPN without high salt for 44 days.

FIG. 6. Edema and some cell reaction are evident. H&E stain. × 95.

FIG. 7. AMPase reaction appears in the stroma as well as in endothelium. × 95.

FIG. 8. ATPase reaction in a section consecutive to that shown in Figure 7. A positive reaction is seen in the valve at cellular tips and in endothelium. The stroma exhibits no reaction. × 95.

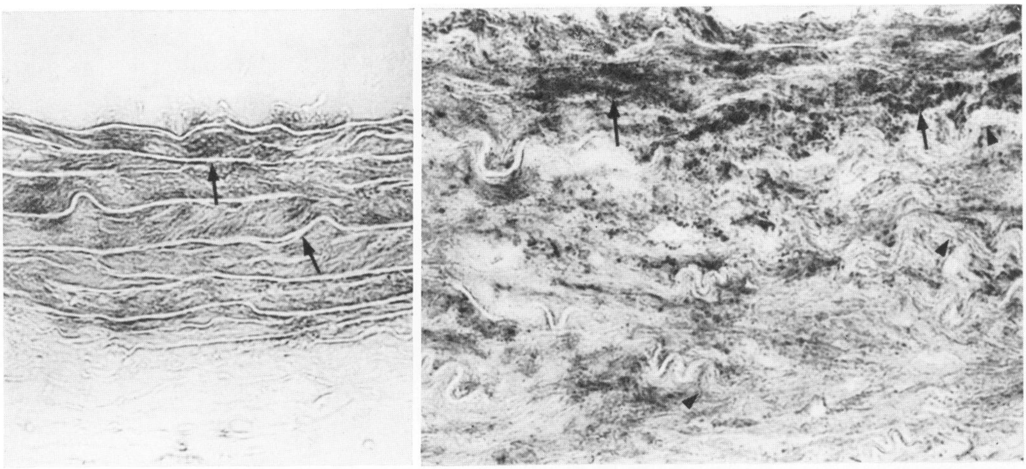


6 7

9 8

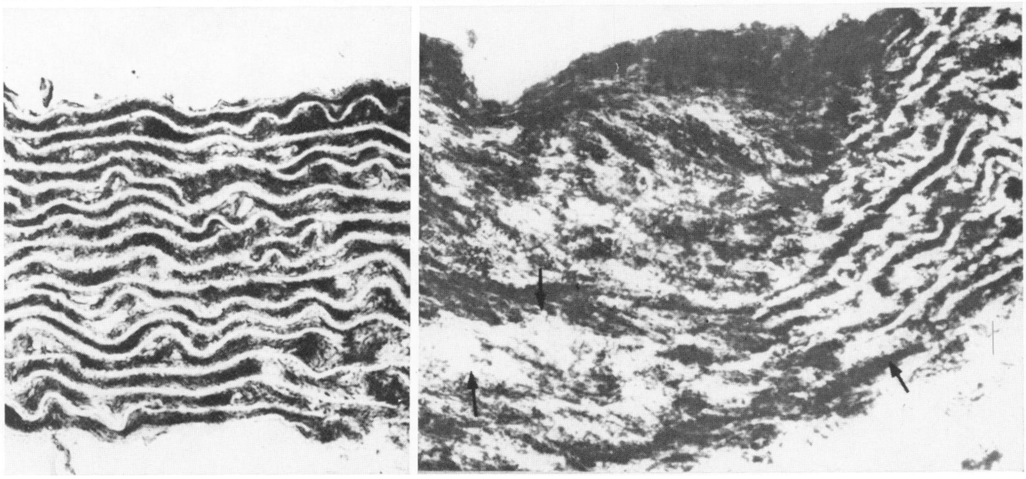
FIG. 9. Alkaline phosphatase reaction in a section consecutive to that shown in Figure 8. A positive reaction appears in the valve stroma. Most reacting cells do not exhibit a positive reaction (arrow). × 95.

10A



10B

11A



11B

Figures 10 to 13 inclusive are enzymatic reactions in the ascending aorta of a rat fed BAPN for 44 days. This portion of aorta was thickened focally but neither dissection nor rupture were found. Figures 10 to 13A and B are from same cross section of the aorta. A, an intact portion, and B, a thickened portion.

FIG. 10A. A weak focally positive AMPase reaction is present between elastic fibers. The elastica is relatively regular and of ordinary structure (arrows). × 235.

B. AMPase reaction is increased focally between altered elastic fibers (arrows). Many parts of the elastica have lost their refractility with evident swelling and separation (arrow heads); only a few undulating fibers are seen. There is no AMPase reaction in such areas. × 235.

FIG. 11A. A strongly positive ATPase reaction is seen between the elastic fibers. The fibers show normal structure except for minor swelling. × 190.

B. A marked increase of ATPase reaction is evident in the altered area. The elastic fibers have lost their ordinary structure and have a patchy pattern (arrows). × 190.

12A 12B 13A 13B

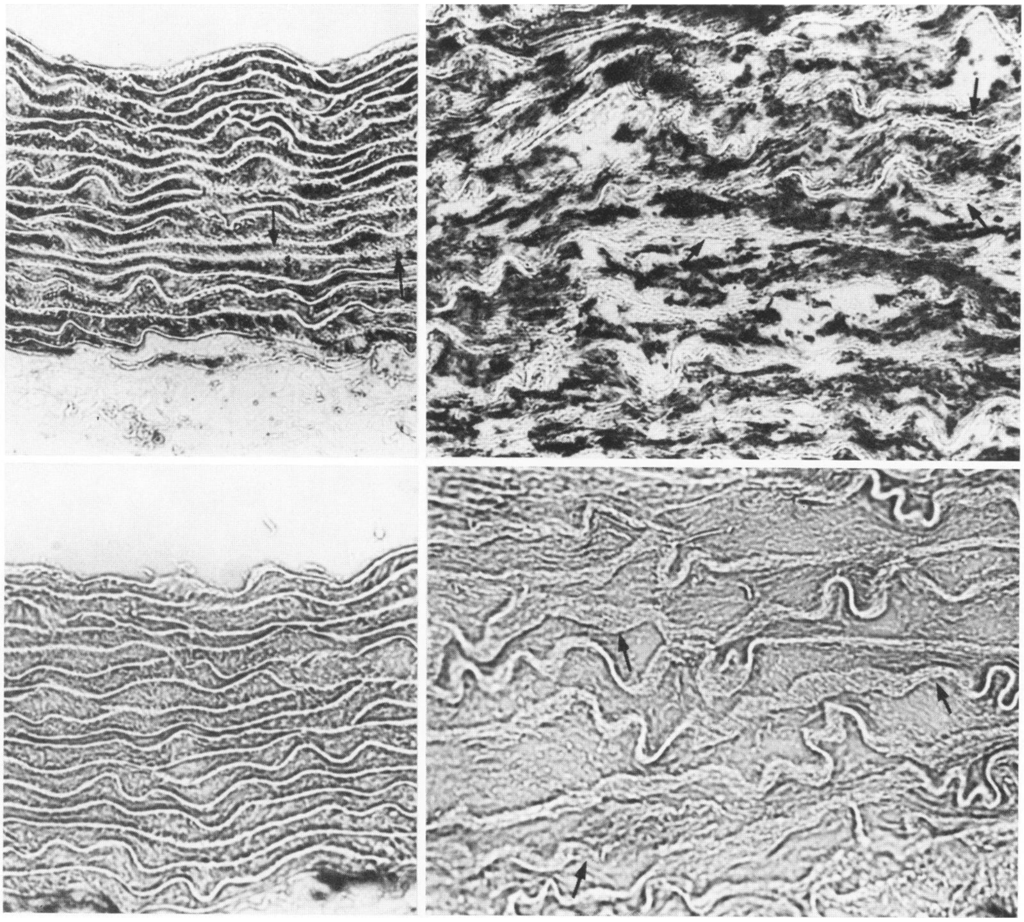


FIG. 12A. Formazan precipitation appears between elastic fibers. With this reaction some elastic fibers exhibit swelling and an irregular granular appearance (arrows) along the margins. × 235.

B. An increased formazan precipitation is irregular between elastic fibers. The fibers have become irregular and swollen and separation is evident (arrows). × 235.

FIG. 13. No alkaline phosphatase reaction is evident in the muscle coat between elastic lamellae.

A. Elastic fibers are relatively well preserved. × 235.

B. Elastic fibers are irregular in structure with swelling, distorted wavy pattern and loss of refractility (arrows). × 235.

Figures 14 to 17. Autoradiograph. L-proline-^{3}H in valve (Figs. 14, 15, 18 and 19) and aorta (Figs. 16 and 17). L-proline-^{3}H, 1 μc per gram body weight was given intraperitoneally and the rat sacrificed 108 hours later. The exposure period was 13 weeks, sections stained with hematoxylin and eosin.

FIG. 14. Mitral valve, control rat. A minimal uptake is seen. × 590.

FIG. 15. Mitral valve, rat fed BAPN for 37 days. Moderate uptake is seen, but there is much more than the control. × 590.

FIG. 16. Ascending aorta, same control rat shown in Figure 14. Minimal uptake is manifest. × 235.

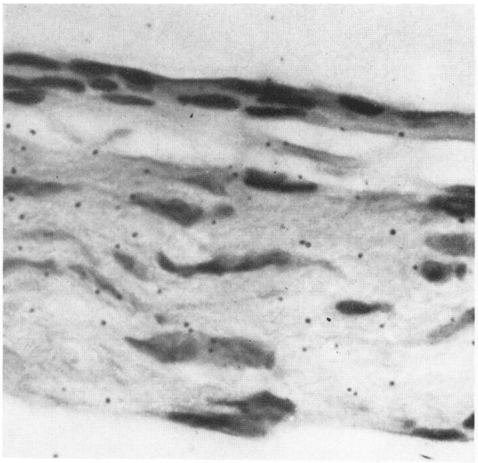
FIG. 17. Ascending aorta, rat fed BAPN in high salt diet for 17 days; dissecting aneurysm and granulation tissue. A fairly good uptake appears, much more than control rat (Fig. 16). × 235.

Figures 18 and 19. L-proline-^{3}H, 1 μc per gram body weight given intraperitoneally and rat sacrificed 48 hours later. The exposure period was 13 weeks, hematoxylin and eosin counterstaining. × 370.

FIG. 18. Mitral valve, control rat. Minimal uptake is shown. × 370.

FIG. 19. Mitral valve, rat fed BAPN and high salt for 34 days. Moderate uptake is shown. × 370.

14



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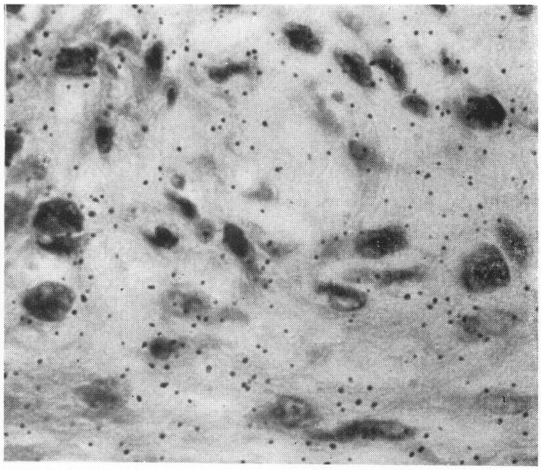

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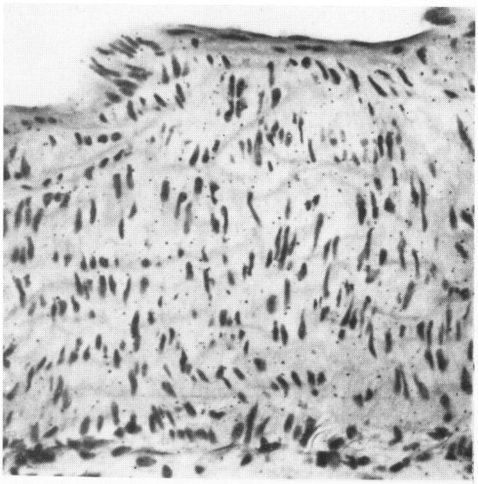

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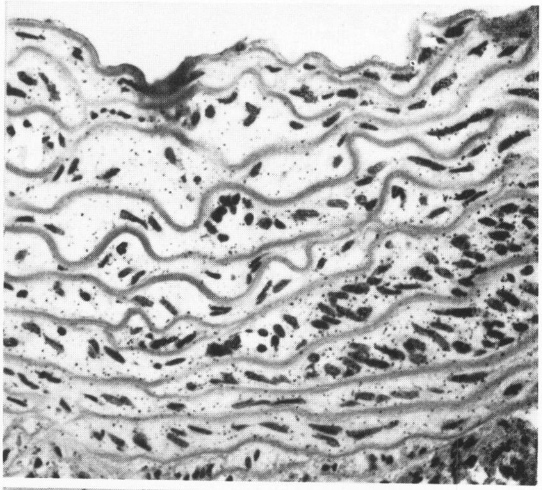

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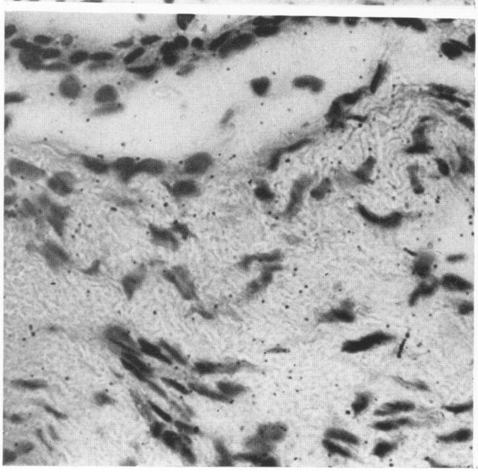

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