Distensibility Characteristics of the Left Ventricle of the Lathyritic Turkey[1] (35308)

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Compared to other muscular organs, there is very little collagen in the left ventricle of the heart. Only about 1% of the wet weight of the left ventricle is due to collagen (1) and most of this collagen is found in the coronary vessel walls and the valvular apparatus. Although there are only a few collagen fibers among the muscular fibers, it is generally assumed that these collagen fibers exert a large effect upon the passive (or diastolic) compliance of the musculature of the left ventricle (2).

Experiments designed to test collagen's contribution to the mechanical properties of the left ventricle have yielded conflicting results. Rabbit hearts incubated in collagenase-containing solutions (1.5 mg/ml) were reported to have increased left ventricular distensibility (3). However, Grimm and Whitehorn (4) bathed beating hearts with solutions containing from 0.003 to 0.20 mg/ml collagenase and concluded that collagen was not responsible for any diastolic mechanical properties of the heart. Both these experiments were attempts to remove collagen *in vitro* and both may be criticized: the first cited used dead hearts; the second used levels of enzyme which might have been inadequate. The experimental protocols would have been improved had the collagen been altered *in vivo* beforehand. A metabolic disease known as "lathyrism" provides a means for altering the collagen beforehand. In many immature laboratory animals, lathyrism can be induced by feeding a lathyrogen such as beta-aminoproprionitrile (BAPN). Lathyritic collagen is weaker than normal and tissues become more extensible (5). The turkey poult is susceptible to lathyrism (6) and its heart is of a size convenient for experimental manipulation. If collagen is important in the passive distensibility characteristics of the left ventricle, and if cardiac collagen is as susceptible to the biochemical lesion as the collagen of other tissues, hearts of lathyritic poults should have markedly altered pre- and postmortem pressure-volume characteristics.

We report here that the distensibilities of lathyritic and untreated turkey hearts were similar just following excision, but that lathyritic hearts were significantly less distensible after *rigor* had developed.

Materials and Methods. The experiments reported below were repeated three times. Each time, 12 to 18 turkey poults of the Broad Breasted white strain were presented a Purina Chick Growena Checkerettes diet to which we had added 0.07% BAPN-furmarate (Aldrich Chemical Co., Milwaukee, Wisconsin). One group was fed the diet beginning at 4 weeks of age; the other two groups were started at 6 weeks of age. Similar numbers of birds were fed the untreated chick diet. When the first poults died after eating the BAPN for 3 to 4 weeks, all the surviving treated birds and the control birds were killed during the subsequent 4 days.

The animals were anesthetized with sodium pentobarbital (30 mg/kg, iv). Heparin (2000 units) was injected through the same needle. The thorax was quickly opened, the pericardial sac was incised, and the heart was removed. A slit extending from the atrioventricular groove almost to the apex was made in the right ventricular wall. The thin, nonseptal wall of the right ventricle, if left intact, would limit the left ventricular movements we wished to study.

A multi-holed, truncated conical cannula

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was inserted into the left ventricle via the ascending aorta. An umbilical tape ligature in the atrioventricular sulcus anchored the cannula and did not allow significant coronary artery perfusion. A Harvard Apparatus Co. pump set to deliver or withdraw 4.12 ml/min was attached to the cannula. Intraventricular pressure changes were measured by a Statham P-23Gb transducer which was attached to a sidearm of the infusion apparatus. A permanent record was made by an Electronics for Medicine DR-8 photographic oscillograph. The infusion fluid was a Ringer–Locke solution. The cannulated heart was suspended in a large, aerated volume of the same solution. The bath temperature was 37°. The hydrostatic level of the bath was maintained constant throughout both infusion and withdrawal.

The protocol for all pressure–volume measurements was as follows: (i) As much fluid as possible was withdrawn from the left ventricle and pressure at this "zero" volume was recorded. (ii) A continuous pressure record was obtained while infusing the left ventricle until the pressure reached 70–80 mm Hg. (iii) The pump was then reversed and fluid was withdrawn at the same rate as infusion. The process was repeated. This constituted the first pressure–volume curve (PV-1). PV-1 was always obtained within 4 min after the heart was excised. In most cases the heart was still beating during the filling and emptying processes. If the heart leaked, the preparation was discarded; we succeeded in obtaining 24 "control" and 36 "lathyritic" PV-1 curves. (iv) After 60 min time lapse (when stiffening was maximal) the second PV curve (PV-2) was recorded. Only a fraction of the volume accommodated by the heart in PV-1 could be accommodated now. (v) Immediately after PV-2 was obtained, the heart was stretched one time by forcibly injecting about 2 ml of the infusion fluid. This procedure stretched the now-dead heart and ruptured some bonds of contractile proteins. (6) PV-3 was then recorded.

In order to estimate the severity of the collagen lesion, the salt-soluble collagen of thigh dermis and foot tendons was estimated by the method of Heikkinen (7). These tissues were removed immediately after the heart was removed.

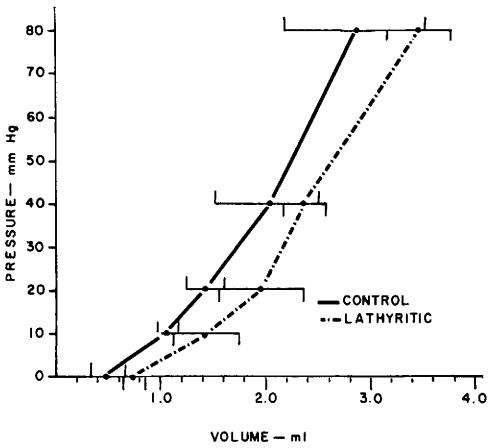

FIG. 1. Mean pressure–volume (PV-1) curves obtained during infusion of freshly-excised normal and lathyritic turkey left ventricles. The horizontal bars represent two standard errors of the mean for each mean value plotted.

Results. The birds showed the expected symptoms of lathyrism (*e.g.*, hock and toe deformities) and their salt-soluble collagen was significantly increased: 13 ± 3 and 6 ± 3 mg/g of tissue wet weight for control skin and tendon, respectively; 60 ± 7 and 43 ± 8 mg/g for lathyritic skin and tendon, respectively. The control and experimental values differed from one another at the 0.01 level of probability.

When we first did the experiment our pressure–volume changes were so different from what we expected that we next tried younger animals, then another group of 6 week-old poults. There were no differences in the results.

PV-1. The typical P-V curve is hysteretical. Figure 1 demonstrates only the infusion portions of curves from 8 control and 11 experimental animals of one experiment. There were no significant differences between the control and experimental groups in either ascending limbs of the complete infusion–withdrawal cycle.

PV-2. The left ventricular chambers of both groups of animals were hardly distensible. Less than 0.5 ml of fluid infused into the hearts raised the pressure to 80 mm Hg.

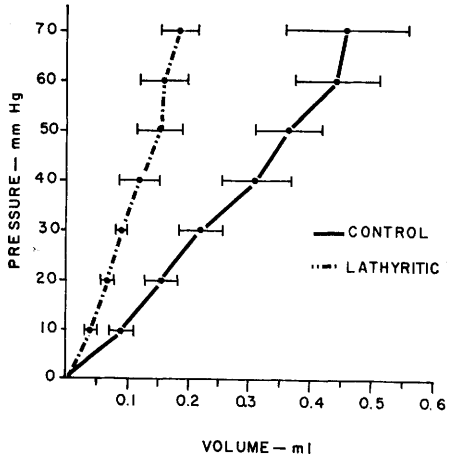

FIG. 2. Mean pressure–volume curves obtained after *rigor* had developed and after forcible stretching of the left ventricular chamber (PV-3). The horizontal bars represent two standard errors of the mean for each mean value plotted.

There was no statistical difference between the two groups.

PV-3. Unexpectedly the left ventricular chambers of the lathyritic group, after first being stretched with about 2 ml of fluid, were more resistant to changes in volume than the control group. Figure 2 summarizes these data.

Discussion. The evidence indicates that the experimental groups of birds were lathyritic: the salt-soluble moiety of skin and tendon collagen was significantly increased; the treated birds showed the typical symptoms; some of the animals died of internal hemorrhage; and their aortas were more extensible (data to be published elsewhere). We had induced a generalized collagen lesion. Whether the ventricular collagen was also affected still remains to be answered. The similarity of the PV-1 data from experimental and control ventricles would suggest: (a) that there is little or no change in the ventricular collagen caused by the disease state; or (b) that collagen is not involved in bearing resting left ventricular tension. Otherwise the distensibility of hearts from lathyritic animals would have been greater than those from the control group.

It is possible that the collagen of the hearts of the BAPN-treated group was not altered to the same extent as the connective tissue elsewhere in the treated bird body. It has recently been demonstrated that physicochemical properties of collagen differ from muscle to muscle (9, 10). Other types of experiments are necessary to settle this point.

Like PV-1, PV-2 showed no differences between the two groups of animals. PV-3, however, did show a significant difference: it took less fluid to reach any given pressure tested for the experimental animals than it did for the control. Thus, hearts from lathyritic birds were significantly less distensible than were the controls. One must conclude that our treatment of the birds affected some passive component of the ventricular fibrillar system.

One could speculate that the BAPN inserts itself between the tropocollagen molecules and inhibits cross-linkages from forming *in vivo*. Exposure to a BAPN-free bathing solution might remove these molecules and permit cross-linking *de novo* thus making a less extensible meshwork.

Although we have emphasized that lathyrism affects primarily collagen, other connective tissue components have also been shown to be altered by lathyritic agents. Changes in acid mucopolysaccharides have been reported (11–13), as well as have changes in elastin (14) and in both groups of substances (15).

1. Chvapil, M., "Physiology of Connective Tissue," 417 pp. Butterworths, London (1967).

2. Buccino, R. A., Harris, E., Spann, Jr., J. F., and Sonnenblick, E. H., Amer. J. Physiol. **216**, 425 (1969).

3. O'Brien, L. J., and Moore, C. M., Experientia **22**, 845 (1966).

4. Grimm, A. F., and Whitehorn, W. V., Amer. J. Physiol. **210**, 1362 (1966).

5. Levene, C. I., and Gross, J., J. Exp. Med. **110**, 771 (1959).

6. Simpson, C. F., Pritchard, W. R., Harms, R. H., and Sautter, J. H., Exp. Mol. Pathol. **1**, 305 (1962).

7. Heikinen, E., Acta Physiol. Scand., Suppl. **317**, (1968).

8. Barnett, B. D., Bird, H. R., Lalich, J. J., and Strong, F. M., Proc. Soc. Exp. Biol. Med. **94**, 67 (1957).

9. Mohr, V., and Bendall, J. R., Nature (London) **223**, 404 (1969).

10. McClain, P. E., Nature (London) **221**, 181 (1969).

11. Bean, W. B., and Ponseti, I. V., Circulation **12**, 185 (1955).

12. Gillman, T., and Hathorn, M., J. Embryol. Exp. Morphol. **6**, 270 (1958).

13. Alper, R., Prior, J. T., and Ruegamer, W. R., J. Atheroscler. Res. **8**, 787 (1968).

14. Ham, K. N., Aust. J. Exp. Biol. Med. Sci. **40**, 353 (1962).

15. Lalich, J. J., and Ishida, K., Arch. Pathol. **82**, 129 (1966).

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