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Aortal Mucopolysaccharide Changes After Epinephrine Administration in Rabbits. (29470)

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Lorenzen(1) reported gross aortic lesions involving medial necrosis and occasional intimal proliferation after 15 days of intravenous epinephrine in rabbits. At the same time, based on hexosamine measurements and assay of the S^{35} content of dried tissue, Lorenzen suggested that the pathosis observed was associated with an accumulation of acid mucopolysaccharide (MPS) and that this accumulation is probably a reflection of reparative processes started by the epinephrine-induced injury.

Lorenzen's data are subject to additional interpretation in that his results also support the hypothesis that epinephrine initiates and aggravates a defect in the "biochemical architecture" of aortal wall which renders it susceptible to degenerative change, and that the defect is manifested by an alteration in aortal MPS metabolism. Complete proof of such a hypothesis would involve rather extensive experimentation, but an important segment of that proof requires that intravenous epinephrine will induce aortal MPS changes prior to, and in the absence of, gross pathosis. Accordingly, it was decided to administer epinephrine to rabbits over a portion of the dosage schedule described by Lorenzen and determine if alterations of MPS metabolism can be identified in the wall of the aorta.

Methods. Twenty-two male and female rabbits of the New Zealand strain (avg wt 4.5 kg) maintained on Rabbit Chow and water *ad lib* were housed in individual cages and divided equally into 2 groups. Group E animals were injected (ear vein) twice daily for 5 days with 0.025 mg/kg of epinephrine in physiological saline. Group C received the saline alone in equivalent volumes on the same schedule. On the evening of the fifth day, each rabbit was given 300 μc of carrier-free $Na_2S^{35}O_4$ in physiological saline by subcutaneous injection and all animals were sacrificed 16 hours later. Examination of aortae at autopsy failed to identify gross pathosis of any sort with the exception of a single intimal lesion in the thoracic aorta of C-5. Aortae were excised, cleaned of adhering fatty tissue and individually digested in a papain EDTA-cysteine-Po_4 buffer mixture (pH 5.5) at 60° for 64 hours. NaOH was added to each solubilized tissue sample to a final concentration of 0.5 N and agitated gently at 4°C for 4 hours. The pH was then adjusted to 5.5 with 5 N HCl and MPS were isolated and reprecipitated to constant S^{35} specific activity by successive separations in 75% ethanol in water. The final precipitate was taken up in 0.25 ml of water and this solution assayed for MPS content(2)* and MPS specific activity.†‡ Electrophoresis of this solution on cellulose acetate strips (formic acid-pyridine buffer, pH 3) demonstrated 2 acridine orange positive components differing only slightly in mobility. The faster of the

* Repeated recovery studies of added glucuronolactone to MPS isolated from epinephrine and control rabbit aortae ranged from 100-110%.

† When 10^4 inorganic S^{35} counts were added to enzymatic digests of non-radioactive rabbit aortae, and the MPS isolated by 3 successive precipitations with 75% ethanol in water, the final precipitates displayed background levels of radioactivity.

‡ Scintillation Counter, Packard Model 314EX.

TABLE I. Aortal MPS Concentrations and Specific Activities in Epinephrine (E) and Saline (C) Injected Rabbits.

No.	MPS specific activity, c/min/10 μg glucuronolactone E	C
1	252	50
2	252	89
3	Died	75
4	250	89
5	304	58
6	260	66
7	240	80
8	255	119
9	375	121
10	414	74
11	330	60
Means	293	80
P*	<.01	

* Mann Whitney Test.

2 moieties possessed a mobility identical to that of a commercial preparation of chondroitin sulfate—the other was not further identified. Analyses demonstrated a nitrogen-uronic acid molar ratio of 2, which suggests that the solute was approximately 85% MPS. No difference between samples isolated from epinephrine and control rabbit aortae with respect to electrophoretic behavior or uronic acid-nitrogen ratio could be recorded.

Results and discussion. The data listed in Table I indicate that the incorporation of S^{35} into aortal MPS is significantly increased after epinephrine administration in rabbits, and that this change is quite independent of gross pathosis. The higher levels of MPS specific activity observed after epinephrine could be due to either: 1) decreased precursor pool size, 2) decreased concentration of aortal MPS, or 3) increased rate of MPS synthesis. It should be noted, however, that although no attempt was made to assure quantitative isolation of MPS in this experiment, amounts obtained from the aortae of epinephrine-treated rabbits were invariably greater than those isolated from control rabbit aortae. It would seem, therefore, that the alteration of aortal MPS metabolism induced by epinephrine involves either the precursor pools or the rate of synthesis. It is hoped that refinement of our analytical techniques will permit further resolution of this point.

The present findings are at variance with the recent report by Hollander(3) wherein epinephrine produced more than a 50% *reduction* of MPS formation in incubation studies with human aortic tissue removed at surgery for vascular disease. The conflicting observations may reflect a species difference, or may be due to the fact that tissue incubated *in vitro* is not under hormonal or neural control. It is also interesting to compare the results of the present experiment with the data reported by Bollet(4) who measured aortal MPS concentrations in rabbits over a time course of cholesterol feeding and was unable to identify an MPS increase until gross lesions were also observed. There seem to be points of similarity between aortic lesions induced by epinephrine and those observed after cholesterol feeding in rabbits. Therefore, it might prove fruitful to determine if increased aortal MPS^{35} specific activities can also be recorded in cholesterol-fed rabbits prior to the development of gross lesions.

Summary. Aortal MPS^{35} specific activities were compared in separate groups of rabbits injected with epinephrine and with physiological saline for 5 days prior to administration of radio sulfate. Significantly increased aortal MPS^{35} specific activities were found in the epinephrine treated animals although the well documented, epinephrine induced aortic lesions were not observed due to the abbreviated period of epinephrine administration. These data demonstrate that epinephrine induces an alteration of aortal MPS metabolism that is independent of gross pathosis. Therefore, the data support the hypothesis that epinephrine induces a defect in the biochemical architecture of the arterial wall which renders it susceptible to degenerative change.

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