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Received August 24, 1959. P.S.E.B.M., 1959, v102.

Epinephrine-Induced Alterations in Connective Tissue of Aortic Wall in Rabbits.* (25277)

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Alterations in rabbit aortae caused by epinephrine were first described by Josué(1). Later on several others have confirmed his results. Summaries have been given by Hueper (2) and Raab(3). Gross alterations have been observed as a response to epinephrine injected intravenously once daily through two weeks. The purpose of this experiment was to examine the influence of epinephrine on the connective-tissue ground substance in the aortic wall. The amorphous ground substance is characterized by its content of acid mucopolysaccharides, sulfated as well as non-sulfated, whereas to a considerable degree, the collagen is characterized by its content of hydroxyproline.

Materials and methods. Twenty white male rabbits were kept on a uniform, sufficient laboratory diet. The experimental animals had epinephrine† intravenously injected daily for 15 days in doses rising from 0.025 mg/kg once a day to 0.050 mg/kg twice a day during the last 10 days. On the 14th day experimental animals and controls were injected with ^{35}S sulfate, 1 mC/kg subcutaneously (carrier-free ^{35}S sulfate,‡ diluted to a suitable volume with a 0.005% solution of sodium sulfate in physiological saline). 48 hours later they were sacrificed with nembutal (ethyl-methylbutyl barbituric acid) injected intravenously. The aorta was removed in its whole length together with the heart. The gross alterations were roughly estimated. The thickness of the walls of the right and left heart ventricle was measured. Preparations for histologic examinations were removed from the thoracic and abdominal aorta. The tissues were fixed in a 4% aqueous solution of lead subacetate and a 4% aqueous solution of neutral formalin. The sections were stained with hematoxylin-eosin (Harris), orcein (Unna) and Sudan III. Further the sections were stained with a ½% aqueous solution of toluidine blue for metachromasia, and for calcium by the method of von Kossa. For the biochemical investigations the thoracic aorta from the aortic valve to the diaphragm, the site of the most pronounced gross alterations, was used. The aorta was cut longitudinally into 2 halves, and the intima plus media were peeled off. The water content was determined as the difference in weight before and after 48 hours drying in an oven at 37°C. The total hexosamine content was determined to estimate the content of acid mucopolysaccharides, by the method of Elson and Morgan(4) as modified by Boas(5). The analysis was carried out on half of the dried and minced tissue by hydrolysis with 2 N HCl as described by Kirk and Dyrbye(6). The sulfated mucopolysaccharides were estimated by the *in vivo* uptake of ^{35}S sulfate as devised by Moltke(7). After drying, the uptake was measured on the hydrolysate of the dried tissue, which was also used for the determination of the hexosamine. The activity was measured with a Geiger-Müller tube having a mica-end window weighing 2.7 mg/sq cm. Determination of the hydroxyproline

* Supported by grants from Reinholdt W. Jorck og Hustrus Fond.

† 0.1% solution of epinephrine hydrochloride.

‡ Obtained from the Radiochemical Centre, Amersham, England.

TABLE I. Gross Alterations in Rabbit Aortae. The water content in % of wet weight, and uptake of ^{35}S sulfate in counts/min./10 mg dried tissue.

Gross alterations*		Water content		^{35}S sulfate uptake	
Exp.	Control	Exp.	Control	Exp.	Control
0	0	71.2	65.5	286	82
4	"	68.1	57.4	322	92
0	"	67.5	69.8	200	124
0	"	72.2	63.8	230	127
2–3	"	68.5	66.7	266	103
1	"	72.7	65.2	155	87
3–4	"	73.2	65.2	204	90
0	"	72.3	62.8	184	90
2	"	69.7	66.3	224	99
2	"	70.2	67.9	140	93
Means		70.6	65.0	221	99
		± 0.7†	± 1.0	± 18	± 5
P value		<.001		<.001	

* Gross alterations were roughly estimated by grading from 0-4.
† Stand. dev. of mean.

should reveal alterations in the amount of collagen. The analysis were made on the remnants of the dried tissue by Neuman and Logan's method (8) as modified by Martin and Axelrod (9).

Results. The gross findings appear from Table I. In 6 out of 10 animals whitish plaques were found scattered chiefly over the thoracic aorta, somewhat less pronounced in the ascending aorta and the arch, and only very few changes could be observed in the abdominal aorta. In one animal the whole thoracic aorta was altered. The lesions brought about a thinning of the wall and a few aneurysms. Thickening of the walls of the heart ventricles was found mainly on the left side. These changes were most pronounced in the animals exhibiting the most marked alterations of the aorta. Furthermore several animals showed effusions in the serous cavities, particularly the pleura and the pericardium.

Microscopically, scattered necrotic areas of media with calcium deposits were found, and in their periphery, accumulations of amorphous ground substance, which stained metachromatically with toluidine blue indicating a content of acid mucopolysaccharide (10). A moderate proliferation of fibroblasts and a fragmentation of the elastic membranes were observed. Microscopic alterations were found also outside the grossly visible lesions. By fat-staining of frozen sections no difference between experimental animals and controls was noted, particularly no deposition of fat near the media lesions. In a few animals a definite proliferation of the intima was observed, especially in areas presenting media necrosis.

Table I shows, that the content of water was somewhat higher in the aortae of the experimental animals than in the controls. The hexosamine values (Table II) showed a significant rise in the experimental animals, whereas the hydroxyproline values were somewhat lower than in the controls, but the difference was not significant. Accordingly the hexosamine/hydroxyproline ratio (Table II) was higher for the experimental animals. The uptake of ^{35}S sulfate is listed in Table I. It appears that the uptake in the experimental animals was considerably greater than in the controls.

Discussion. The observations of gross and microscopic alterations in aortae accord with previous investigations. The increased uptake of ^{35}S sulfate and the increased water content together with the findings of metachromasia of the ground substance and the hexosamine increase suggest an accumulation of acid mucopolysaccharide. 24-48 hours after peritoneal injection the main part of ^{35}S sulfate is likely to be found esterified in chondroitin sulfuric acid in different tissues, among others the aorta (11). Among the acid mucopolysaccharides, hyaluronic acid has a

TABLE II. Content of Hexosamine and Hydroxyproline in Rabbit Aortae, μg/mg Dried Tissue and the Hexosamine/Hydroxyproline Ratio.

He		Hp		He/Hp	
Exp.	Control	Exp.	Control	Exp.	Control
6.7		14.2	15.4	.47	
7.2	7.1	15.1	19.5	.48	.36
8.5	6.9	13.2	14.6	.64	.47
8.6	6.4	18.3	16.6	.47	.39
7.0	6.4	15.2	18.1	.46	.35
6.1	6.0	17.3	20.2	.35	.30
7.4	6.1	16.3	23.4	.45	.26
7.4	4.8	21.7	19.2	.34	.25
6.7	5.3	15.9	14.5	.42	.37
6.4	5.7	16.8	20.7	.48	.28
7.2	6.1	16.4	18.2	.45	.34
±.2*	±.2	±.8	± 1.0	±.03	±.02
		P value			
<.01		<.10		<.01	

* Mean ± stand. dev. of mean.

considerable water-binding capacity. In human aortae the greater part of the mucopolysaccharide fraction is chondroitin sulfuric acid but other mucopolysaccharides including hyaluronic acid seem to be present(12). While the hydroxyproline content showed no definite change, there was an increase in the hexosamine/hydroxyproline ratio indicating an increase in the amount of amorphous ground substance in proportion to the collagen.

The mechanism of the epinephrine effect is not clear. Theories have been advanced of vascular injury as a result of the variations in blood pressure accompanying the epinephrine injections; of an anoxic injury via the vasa vasorum; and of a direct effect on the cellular metabolism. The accumulations of acid mucopolysaccharides noted in these experiments is probably a reflection of reparative processes started by the epinephrine induced injury. Healing wounds contain large amounts of acid mucopolysaccharides, and the increased uptake of ^{35}S sulfate accord with previous observations of wound healing (13,14).

Summary. Alterations in rabbit aortae were brought about by intravenous administration of epinephrine. Microscopic examination revealed accumulation of metachromatic ground substance. The aortic content of hexosamine and water together with the uptake of ^{35}S sulfate was increased, whereas the amount of hydroxyproline was unaltered. The increase in the mucopolysaccharide/collagen ratio may indicate a reparative process.

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Received August 24, 1959. P.S.E.B.M., 1959, v102.

Local Morphological Response Following a Single Subcutaneous Injection of Carrageenin in the Rat. (25278)

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Various polysaccharides, mostly of bacterial origin, can induce inflammatory conditions in experimental animals(1,2,3,4). Subcutaneous injections of carrageenin, a polygalactose-sulfate of plant origin, have been used in different species for biochemical analysis of collagen formation(5,6,7,8). The present study of morphological response to a single subcutaneous injection of carrageenin in the rat was carried out to determine whether this plant material could be used as a stimulus to induce an inflammatory lesion.

Material and methods. Seven groups of 6 female Sherman rats (body-weight range 140 ± 10 g) were housed individually and fed Purina Laboratory Chow and tap water *ad lib.* All animals received a single subcutaneous injection of 0.5 ml of a 2% aqueous solution of carrageenin† (molecular weight 100,000 to 500,000 g/mol), on mid-line of back, halfway

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† Obtained through courtesy of Mr. Leonard Stolloff, Seaplant Corp., New Bedford, Mass.