Histochemical Evaluation of Canine Coronary Artery and Aortic Lesions Induced by Intravenous Allylamine

By L. L. Conrad, M.D., I. E. Gonzalez, Ph.D., W. Joel, M.D. and R. H. Furman, M.D.

The early lesions of the coronary arteries and aorta in animals given intravenous allylamine are described and are noted to be characterized by glycogen accumulation and by ground substance change indicated by increased PAS-positive material present after incubation with diastase. It is suggested that heterogeneous polysaccharides and/or substances containing 1-2 glycol linkages, other than sulphated acid polysaccharides, may be of importance in the early lesion.

When experimental vascular injury is produced by any one of a variety of agents and is followed by alteration in the nature and/or amount of blood lipids, vascular lesions containing lipid may result which are often designated "atherosclerotic." This study was undertaken to ascertain the progressive manifestations and histochemical alterations characterizing the early allylamine vascular lesions in otherwise "normal" dogs. Other than for preliminary communications, a histochemical study of allylamine-induced vascular lesions has not been reported.

Allylamine (CH₂:CHCH₂NH₂) was demonstrated to produce capillary damage by Eppingei in 1935, and shortly thereafter Mellon described intramural vascular hemorrhage followed by proliferative endarteritis and nodular periarteritis at the site of intracutaneous allylamine injection in rabbits. Waters subsequently noted fibrinoid necrosis, principally of medium sized and smaller coronary arteries, following intravenous allylamine in dogs.

PROCEDURE

Twenty-six mongrel dogs of both sexes and varying ages were given allylamine intravenously as a 1 per cent solution neutralized with HCl in doses varying from 16 to 26 mg./Kg. Most animals received one to three injections at intervals of 24 to 48 hours with death or sacrifice 12 hours to 8 days after the last injection. Two animals were sacrificed 7 days after a series of 8 allylamine injections at 48 hour intervals. Eight of the animals received 1 to 7 intravenous injections of egg yolk suspension in an attempt to induce lipid deposition in the vascular lesions. Two of these animals received 6 and 7 injections of saline egg yolk suspension and were sacrificed at the end of 14 and 13 days respectively. Six of the treated animals received daily oral cholesterol, 1.5 Gm./Kg. In addition, three of the animals receiving egg yolk and three of the six fed cholesterol received daily heparin,* 2 mg./Kg. i.v.

Eighteen untreated animals served as controls.

Sections of the heart, coronary arteries, aorta, lungs, liver, kidney, and spleen were obtained from all animals, fixed in formalin and embedded in paraffin.

Routine histologic sections were stained with hematoxylin and eosin, Van Gieson-AVeigert's iron hematoxylin and Orcein (Kornhauser). Histochemical technic included periodic-acid-Schiff (McManus technic), Hale (Rinehart modification), toluidin blue (metachromasia) and alcian blue (Pearse).

RESULTS

The earliest changes seen were those in animals sacrificed or dying 10 to 18 hours after a single injection of allylamine. Edema of the innermost portion of the media and swollen medial cells of small epicardial and penetrating coronary branches were frequent early manifestations of arterial injury. Occasional "smudgy" areas were detected in the hematoxylin and eosin sections and were associated

* The heparin for this study was generously supplied by the Upjohn Company of Kalamazoo, Michigan.
with a positive Schiff reaction, although these changes were minimal. In the animals receiving 3 injections of allylamine daily or every other day and sacrificed one week after the last injection, the changes were much more definite and in some instances were striking (figs. 3–6). In most instances the internal elastic membrane was thickened and exhibited an exaggerated PAS reaction. The wall of the coronary arteries was swollen and edematous, particularly those in the papillary muscles of the left ventricle and atrium. There was loss of cytoarchitectural detail in the media, and the nuclei were vesicular. At the site of vascular injury necrosis and karyorrhexis were often evident, as well as partial hyalinization of the media and intima (figs. 1, 2). The site of injury exhibited an intense PAS reaction (figs. 2, 4). Although some glycogen granules were seen in the arterial wall, incubation of tissue sections in saliva and/or a diastase buffered medium failed to reduce significantly the intensity of the PAS reaction, indicating that glycogen was not the principal Schiff-positive substance. Early lesions noted to be PAS-positive failed to stain with toluidin blue, alcian blue, or with the Hale reaction. However, the appearance of metachromasia and alcian blue and Hale staining substance were noted occasionally, particularly at the fringe of the older medial lesions (figs. 1, 5 and 6); the extent and degree of the reaction were not equally developed in every animal.

The major coronary vessels usually showed only a slight endothelial swelling or thickening without excessive intimal proliferation. There was an increased cellularity of the adventitia and adjacent tissue. In the arteries of the right atrium all the described changes were seen.

In the arch, thoracic and abdominal portions of the aorta the major change discerned was a slight edema of the intima. In many instances the endothelial nuclei of the vasa vasorum appeared swollen and the endothelium of these vessels was thickened. Interstitial edema around these small intramural arteries was evident.

**Fig. 1.** Epicardial coronary artery of the left ventricle of a male dog which had received two injections of a 1 per cent solution of allylamine and two injections of an egg yolk suspension. Karyorrhexis, pyknosis and edema are evident in the wall of the vessel. A few foam cells are visible in this area. Acid mucopolysaccharides should stain blue with this histochemical method. In this vessel the acid polysaccharides appear to be confined to the periphery of the arterial wall. They are not evident at the site of injury. Hale stain. X 75.

**Fig. 2.** The same artery depicted in figure 1, stained by the periodic acid Schiff technique. The site of arterial injury is clearly indicated by the intense PAS stain. Karyorrhexis, pyknosis and early hyaline formation are evident in the subintimal tissue. X 75.

**Fig. 3.** An intramural coronary artery of the right ventricle of a male dog which had received three injections of a 1 per cent solution of allylamine. There is swelling of the endothelial nuclei, subintimal accumulation of an eosinophilic homogeneous substance, and an edematous appearance of the arterial wall that characterize the early allylamine lesion. Hematoxylin and eosin. X 150.

**Fig. 4.** A periodic acid Schiff preparation of the same intramural artery depicted in figure 3, stained after incubation in a buffered diastase solution. The intense staining of the subintimal mucoid substance after diastase incubation excludes glycogen as the PAS-positive substance. X 150.

**Fig. 5.** This is the same vessel seen in figures 3 and 4. An alcian blue preparation counterstained with neutral red. Note that alcian blue does not stain the subintimal mucoid substance in the allylamine injured artery. The distribution of the sulphated mucopolysaccharides is similar to that seen in figure 6. X 150.

**Fig. 6.** The same intramural vessel depicted in figures 3, 4 and 5, stained with toluidin blue buffered at pH 4. There is thickening and metachromasia of the internal elastic membrane and absence of metachromasia in the subintimal mucoid substance. The sulphated acid polysaccharides appear to be confined to the peripheral portion of the edematous arterial wall. X 150.

**Fig. 7.** Liver of a dog which had received three intravenous injections of a 1 per cent solution of allylamine. The periphery of the lobules and the areas surrounding the portal tetrad are depleted of glycogen. PAS stain. X 75.

**Fig. 8.** Kidney of an allylamine-treated dog. The glomerular capsule and basement membrane of injured renal tubules stain intensely by the periodic acid Schiff technique. PAS positive casts were also noted. X 150.
EVALUATION OF CORONARY AND AORTIC LESIONS

Vascular changes in other viscera were also noted. A hyperemic liver, portal veins distended with blood and thickened, edematous walls of the hepatic arteries, with extravasation of blood in the region of the portal tetrad, characterized the injury in this organ. There was a striking decrease of glycogen noted in the periphery of the hepatic lobules and in the region of the portal canals (fig. 7). Those hepatic cells which were depleted of glycogen, after toluidin blue staining, exhibited a moderate metachromasia. Numerous brilliantly metachromatic mast cells were present in the wall of the central vein, in the portal vessels and in the perivascular connective tissue.

The most conspicuous changes noted in the kidney were thickening of the glomcrular capsule and thickening of the basement membrane of numerous tubules. Both stained intensely with PAS. PAS-positive casts were noted in many tubules. Frequently the glomeruli were strongly PAS-positive but failed to stain with alcian or toluidin blue (fig. 8).

In two animals sacrificed three weeks after eight allylamine injections the changes were minimal. There were no demonstrable lesions of the arteries of the papillary muscles of the left ventricle or of the penetrating vessels of the right ventricle. In the larger coronary arteries there was some increase in Hale staining material and an increase in elastic fibers with duplication of the elastic membrane. Small foci of myocardial necrosis manifest by deep PAS staining and loss of cytoarchitectural detail were frequently present.

The administration of intravenous egg yolk suspension or oral cholesterol supplementation, with or without concomitant heparin administration, produced no evidence of lipid deposition which could be detected by Oil-Red-O staining. The vascular lesions in these animals did not differ from those noted in animals receiving allylamine alone.

Allylamine injection, alone or together with intravenous egg yolk or oral cholesterol administration, did not alter significantly the serum total or free cholesterol levels or serum phospholipid values in seven animals in which these serial determinations were made. In two animals receiving only allylamine no change in the serum lipoprotein distribution, as determined ultracentrifugally utilizing a solvent density of 1.21 Gm./ml. occurred, nor were there changes in serum cholesterol or phospholipid concentrations.

DISCUSSION

Of the various factors considered in “atherogenesis” and coming under experimental study, alteration of the vessel wall ground substance has received comparatively little attention. Exhaustive studies of the histogenesis of human coronary and aortic arteriosclerosis by Faber, Moon and Rinehart, and Taylor indicate that ground substance change is an important characteristic of the early lesion. Our studies indicate that ground substance alteration is a consistent phenomenon in the allylamine-treated dog. However, the degree and extent of this change was not equally developed in every animal.

Prevailing studies indicate that acid mucopolysaccharide alterations are of paramount importance in experimental arteriosclerosis. Our observations suggest that in the early allylamine lesion the most significant lesions are PAS positive but fail to stain with toluidin blue, alcian blue or with Hale stain. The latter observation suggests that ground substance alterations resulting from allylamine administration may not be ascribed entirely to sulfated acid polysaccharides and that the amorphous continuum separating cells and fibers of injured arteries may contain heterogeneous polysaccharides or other substances possessing 1-2 glycol linkages that may be of primary importance in the early development of arterial lesions.

These studies emphasize the need for a clearer characterization of the mucoid alterations and/or reconstitution of the polysaccharides when tissue injury occurs.

Our failure to observe significant lipid deposition or more than a few foam cells in the eight animals in whom the injection of a saline egg yolk suspension was combined with allylamine is surprising in view of Waters' publications, but may be related to an insufficient elapse of time, since all were sacrificed on or before 14 days after beginning treatment. Studies by
Wilens and McCluskey of the permeability of excised arteries to serum lipid suggest that lipid deposition at the site of ground substance change might be anticipated in the allylamine treated animal, since they noted increased affinity of medial tissue for PAS stain when lipid retained in the media was removed by fat solvent. The possibility exists, however, in their studies, that ground substance change was secondary to lipid accumulation.

SUMMARY AND CONCLUSIONS

The early lesions of the coronary arteries and aorta in the allylamine treated dog are described and noted to be characterized by the accumulation of glycogen and by ground substance change, evidenced by increased PAS-positive material noted after incubation with diastase. Our observations suggest that heterogeneous polysaccharides and/or substances containing 1-2 glycol linkages, other than sulphated acid polysaccharides, may be of primary importance in early arterial lesions. Lipid deposition at the site of ground substance change was not noted to be a feature of this lesion, even when saline yolk suspension was repeatedly administered intravenously.

SUMMARY IN INTERLINGUA

Es describite le prime lesiones de arteria coronari e aorta in canes tractate con allylamine. Iste lesions es characterisate per le accumulation de glycogeno e alteraciones del substantia fundamental que se manifesta in le augmento del materia positiva a acido paraaminosalicylic, que es notate post incubation con diastase. Nostre observationes suggere que heterogenee polysaccharidos e/o substantias continent ligamines glycolic 1-2 (altere que polysaccharidos acide sulfatate) es possibilemente de importancia primari in le prime lesions arterial. Depositos lipide al sito del alteraciones de substantia fundamental non esseva observate como caracteristica de iste lesion, mesmo si repetite administrationes intravenose de suspension salin de vitello esseva effectuate.

REFERENCES


Histochemical Evaluation of Canine Coronary Artery and Aortic Lesions Induced by Intravenous Allylamine

L. L. CONRAD, I. E. GONZALEZ, W. JOEL, and R. H. FURMAN

Circ Res. 1956;4:263-267
doi: 10.1161/01.RES.4.3.263

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1956 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/4/3/263

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/