

Summary. Three chelating agents of the polyamino polycarboxylic acid type, ethylenediaminetetraacetic acid, diethylenetriaminepentaacetic acid, and ethylene bis(α -imino-*o*-hydroxyphenylacetic acid) have been compared in treatment of acute experimental manganese poisoning. EBAA had no therapeutic value, whereas both EDTA and DTPA provided significant protection to rats given lethal amounts of manganese gluconate. It is shown that DTPA is somewhat more effective than EDTA.

The authors wish to thank Mr. William Westfall and Mrs. Elizabeth Moretti for technical assistance.

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Received December 1, 1958. P.S.E.B.M., 1959, v100.

Coronary Arteriosclerosis and Thrombosis in the Rat.* (24701)

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Severe arteriosclerosis has been produced in female rats (Sprague-Dawley) bred repeatedly, unilaterally nephrectomized, and then given ACTH for 4 to 7 weeks (Wexler and Miller, 1). Lesions involve systemic, cerebral and peripheral arteries. Large, flame-shaped hemorrhages and aneurysms are seen in the aortic arch. Particularly conspicuous upon gross examination are the distended and tortuous coronary vessels. Myocardial infarcts are readily visible. Histologically, a spectrum of early to advanced lesions has been observed in coronary arteries. These findings appear to have a special importance because (a) the rat is ordinarily resistant to development of arteriosclerosis(2-5), (b) these rats were not given fat in their diet and had normal serum cholesterol levels, and (c) the pathology in

coronary arteries shows some striking resemblances to that observed in human disease.

Materials and methods. Female rats of the Sprague-Dawley strain were (1) bred repeatedly, (2) subjected to unilateral nephrectomy, and (3) given $\frac{1}{3}$ unit of ACTH (gel)/100 g body weight, subcutaneously, 3 times/week, for 4 to 7 weeks. The particular strain of rat can be as important as the other 3 factors in production of satisfactory arteriosclerotic lesions. After nephrectomy, animals were allowed 10 days to recover before administration of ACTH. Animals were housed in air-conditioned quarters and given *ad lib.* a regular commercial rat chow and water. No extra fat or salt was added to the diet. At autopsy, the rats were decapitated, exsanguinated, and tissues intended for microscopic examination quickly removed and fixed in 10% buffered neutral formalin (Lillie). Hematoxylin and

* This work was supported, in part, by a Grant from U.S.P.H.S.

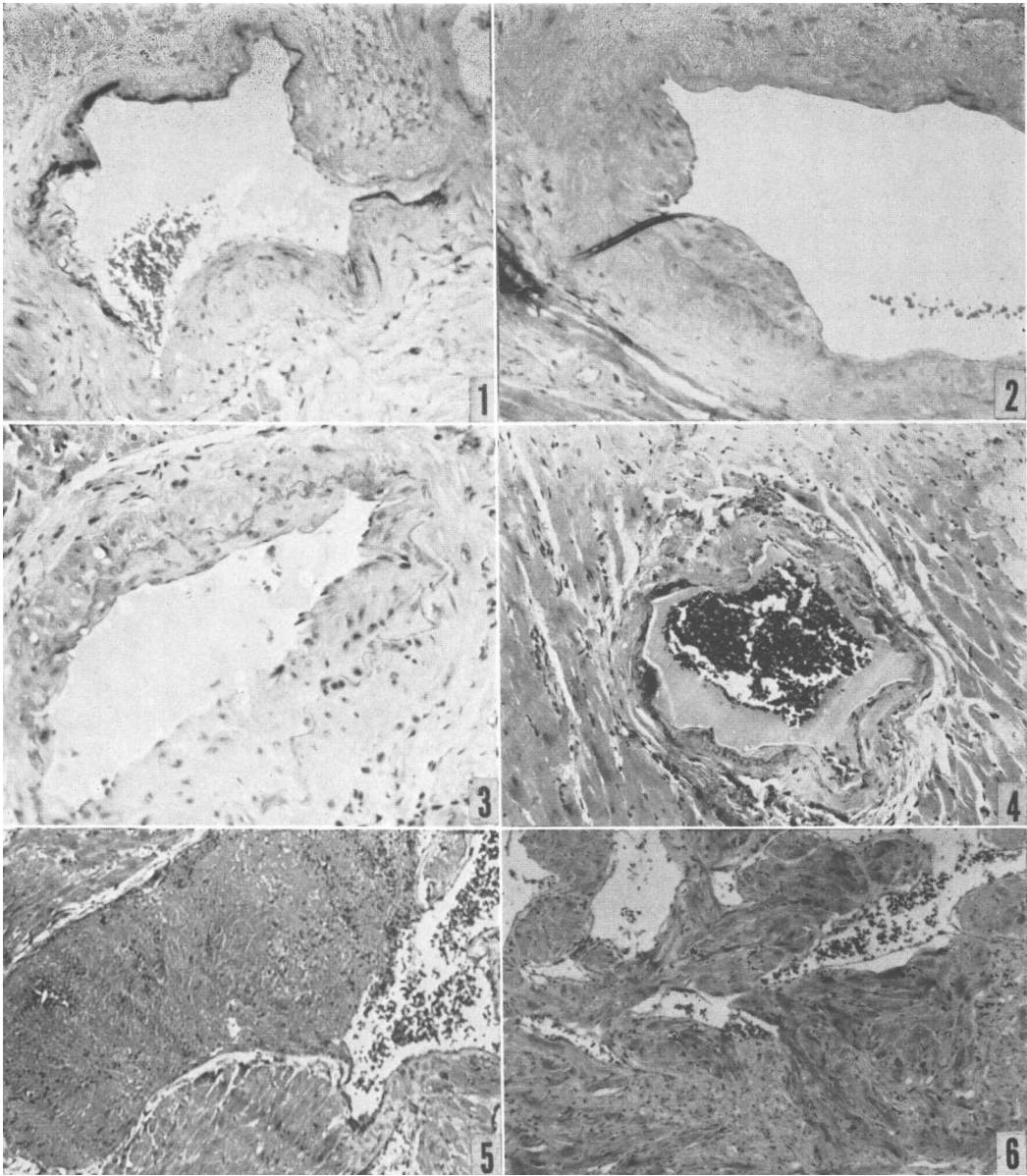


FIG. 1. Main coronary artery and branch showing intimal basophilia and thickening. Female, Sprague-Dawley rat, bred repeatedly, unilaterally nephrectomized, and given ACTH for 7 wk. H & E, $\times 100$.

FIG. 2. Coronary artery of rat treated in same manner showing intimal hyperplasia. H & E, $\times 500$.

FIG. 3. Coronary artery showing more advanced intimal hyperplasia and medial elastosis. H & E, $\times 180$.

FIG. 4. Coronary artery showing early thrombus formation, and intimal and medial basophilia and elastosis. H & E, $\times 210$.

FIG. 5. Mural thrombus formed in atrial wall. Note beginning organization. Myocardial fibers are enlarged. H & E, $\times 100$.

FIG. 6. Myocardial infarct. Light staining areas in lower center and the extreme right represent scarred tissue. Infarcts are most frequently found on endocardial surface of the left ventricle. H & E, $\times 100$.

eosin, Masson and Mallory's connective tissue stain, P.A.S., and Gomori's aldehyde fuchsin stain for elastic fibers were used routinely. Sudan Orange and Sudan Black B were employed for lipids, on frozen sections.

Results. Of 100 rats examined, 75 showed some degree of atherosclerosis of coronary arteries. In this same group, 38 animals displayed significant intimal thickening and derangement of ground substance and connective tissue elements in both intima and media. Similarly, 30 animals manifested myocardial infarction. Microscopic examination showed generalized distribution of arteriosclerotic lesions in the coronary arteries. The larger epicardial branches were most severely involved. Fig. 1 is a cross section of a main coronary vessel and one of its branches, each showing equal involvement. Some lesions consisted of intimal hyperplasia only (Fig. 2) while others displayed intimal basophilia either alone or accompanied by medial elastosis (Fig. 3). We suspect that this basophilic material may be either calcium or mucopolysaccharide material—or a mixture of the two. Thrombi have been found more frequently in medium-sized coronary arteries and less frequently in larger arteries (Fig. 4). The clots exhibited typical appearance of ante-mortem thromboses. Mural thrombi in various stages of organization (Fig. 5) were frequently observed adherent to the atrial wall.

In other areas, coronary arteries show a distinctly different pattern of change. For example, some arteries undergo intimal and medial swelling which caused virtually complete obliteration of the lumen (Fig. 7). This usually occurs in medium-sized arteries. Occasionally, the obliterative type of ingrowth contains giant foam cells (Fig. 8).

Discussion. Virtually all rats so treated developed systemic arteriosclerosis. Approximately 75% of these rats developed coronary lesions. The high incidence of coronary involvement should make this a useful tool in evaluation of preventive and therapeutic procedures. There are many resemblances between the lesions observed in these rats and in humans. Particularly gratifying has been the high rate of true ante-mortem thrombosis with attendant myocardial infarctions. The

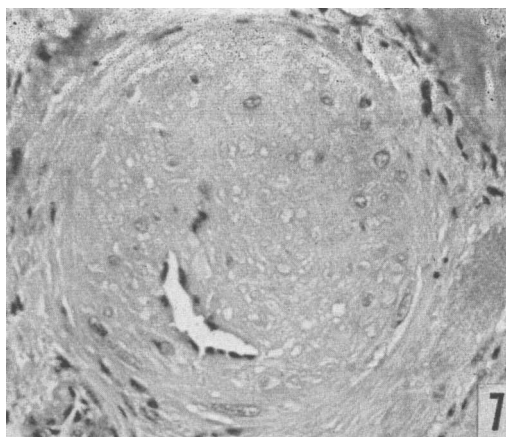


FIG. 7. Coronary artery showing endothelial and medial swelling leading to obliteration of lumen. H & E, $\times 180$.

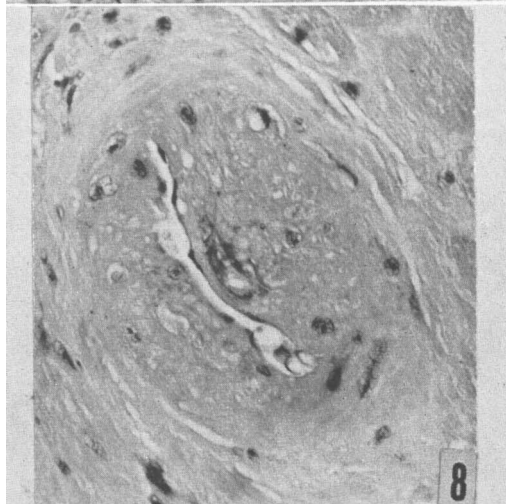


FIG. 8. Coronary artery. Lumen is closed. Note giant foam cells. H & E, $\times 180$.

mural thrombi also closely resemble those in human disease.

The absence of added fat in the diet in our experiments should be noted. Also, there was no elevation in blood cholesterol. However, there may have been changes in other lipids and lipoproteins. Some of these factors are now under study. Only an occasional lesion, and usually an advanced one, showed demonstrable fat (Fig. 8). This condition is the exception rather than the rule.

Sex differences are especially prominent in this form of generalized arteriosclerosis which appears much more readily in the female. For this reason we have restricted this study to

female rats. However, recent observations suggest that male rats have a high incidence of certain types of cardiac lesions (to be reported later).

There are interesting strain differences in appearance of both coronary and general arteriosclerotic lesions. To date, selected strains were more susceptible than others. This is suggestive of the same type of situation existing in the human disease, which is known to have a predilection for certain families.

The intimal basophilia observed in some coronary arteries remains to be studied further. We hope, by histochemical means, to demonstrate whether this is calcium or mucopolysaccharide, or perhaps both existing in close association. Moon(6) has described intimal accumulation of acid mucopolysaccharide as one of the earliest changes in human arteriosclerosis.

Stress[†] would appear to be an important determinant in coronary lesions produced in rats. Three factors appear to be required: repeated breeding, unilateral nephrectomy, and administration of ACTH. It is our supposition that repeated breeding puts stress on the adrenal glands and that this stress is further heightened by administration of repeated doses of ACTH. Possibly, the unilateral nephrectomy intensifies action of ACTH. The parallelism between experi-

mental results and those reported by Friedman *et al.*, in humans(7) is interesting and suggestive.

Summary. 1) Severe coronary arteriosclerosis has been produced in female rats (a) bred repeatedly, (b) subjected to unilateral nephrectomy, and (c) treated with ACTH. The coronary lesions in these animals resemble those seen in man. Morphologically, the coronary arteriopathy is varied, *i.e.*, intimal hyperplasia, deposition of basophilic material in intimal and medial layers (possibly acid mucopolysaccharides), elastosis, and mural swelling leading to complete obliteration of the lumen. Coronary and mural thrombi occur frequently. 2) No fat or salt is added to the diet. Serum cholesterol levels are normal. The possible role of "stress" in induction of this type of coronary artery disease has been discussed.

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Received December 8, 1958. P.S.E.B.M., 1959, v100.

Effect of Intravenous Amino Acids on Plasma Non-Esterified Fatty Acids.* (24702)

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Plasma levels of non-esterified fatty acids (NEFA) seem determined largely by rate at which adipose tissue releases these substances.

* Supported in part by Grant from Nat. Inst. of Arthritis and Metabolic Diseases, Nutrition Fn., N. Y. City and Mead-Johnson and Co., Evansville, Ind.

The following operations result in decrease in concentration of circulating NEFA: Carbohydrate administration(1,2), insulin injection (1), tolbutamide administration(3), and glucagon injection(4). In contrast, increased plasma NEFA concentrations have been ob-