Coronary Atherosclerosis in Renal Hypertensive Rats. (27424)

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Large scale epidemiological studies in man have shown that elevated blood pressure in life is statistically related to necropsy findings of coronary artery disease, atherosclerosis, and myocardial infarction.

Hypertension in experimental animals can be related to atherosclerosis as shown by the fact that experimental aortic coarctation in dogs on an atherogenic diet resulted in greater plaque formation in the vascular areas subjected to hypertension than elsewhere(1). Another factor commonly implicated in cardiovascular atherosclerotic disease is hypercholesterolemia, although the role of cholesterol is poorly understood. It is quite true, however, that atherosclerosis can be produced in several species by feeding diets rich in cholesterol.

To shed light on the roles of both cholesterol and induced hypertension in atherosclerosis production, the present experiment was undertaken. Results obtained by the combination of induced hypertension and a diet high in fat and protein but relatively low in cholesterol are presented here.

Procedure. The Grollman procedure(2) was used to produce renal hypertension in adult male Wistar rats, weighing 200-300 g. Briefly, one kidney was removed and the other firmly bound by several turns (a figure 8 wrap) of suture thread. Within 2 weeks the blood pressure was elevated. Since rats are omnivorous and will eat meat readily, an all-meat diet was selected, consisting of ground lean beef fortified with 100 g vitamin mixture† and 227 g Hegsted salt mixture per 10 lb of meat. The food was prepared in 90-100 lb batches, frozen in packets of 2-4 lb wrapped in aluminum foil and thawed as needed. The rats were fed twice daily with amounts just slightly in excess of what they would consume (approx. 40-50 g/day). Analyses of the diet revealed a loss on drying of 53-59% and extractable lipids of 21-29% on a wet weight basis. Total nitrogen ranged from 3.0-3.8% and total cholesterol varied from 57-68 mg% (also wet weight basis). Hypertensive and parallel normal control rats were started on the meat diet within one week of the surgical procedure. Blood pressures were taken shortly after operation and just prior to sacrifice with a photoelectric tensometer,‡ using the right hind foot. All cholesterol values were obtained by the Abell (3) method. At autopsy tissues for histological examination were preserved in 10% aqueous formalin. Frozen sections of hearts were stained with Oil-Red-O and examined microscopically. Aortas were stained and examined grossly as well as histologically. Chow-fed hypertensive and normal controls were also studied for comparison.

Results. Data in Table I show that normal chow-fed rats, intact meat-fed rats and operated chow-fed rats have normal serum cholesterol values and no coronary atherosclerosis. In the operated animals, though all were hypertensive, only the meat-fed animals developed hypercholesterolemia and coronary atherosclerosis. No aortic changes were found in any of the rats, even at 50 weeks on the meat diet. The sharp contrast between the intact and hypertensive meat-fed animals is shown in the Oil-Red-O stained frozen sections of the hearts in Fig. 1-6. No heart sections from the chow-fed rats are shown as all were quite normal. The coronary arteries pictured are from the region of the left heart close to the outer edge of the cross-sections taken approximately ⅓ of the distance down from the top of the heart. The lesions ap-

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† Vitamin Fortification Mixture and Hegsted salt mixture were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

‡ Model #ME-5000 photoelectric tensometer from Metro Industries, Mineola, L. I.

TABLE I. Terminal Data.

Group	Rat #	Exp. period, wk	Body wt, g	Blood pressure, mmHg	Serum cholesterol, mg %	Coronary atherosclerosis*
Intact, chow-fed	50	20	520	136	51	—
	51	20	609	140	65	—
	53	20	422	142	64	—
	54	20	550	132	56	—
Operated, chow-fed	24	20	500	180	68	—
	25	20	467	140	87	—
	31	20	456	240	50	—
	36	20	486	174	76	—
Intact, meat-fed	70	7	—	119	60	—
	75	16	365	116	67	—
	77	26	495	130	65	—
	71	50	504	108	86	—
	74	50	558	130	60	—
	76	50	696	119	78	—
Operated, meat-fed	33	7	350	139	76	—
	34	7	388	119†	99	—
	36	7	394	158	85	—
	35	16	379	180	267	+
	40	16	453	144	150	+
	41	26	390	175	540	+
	46	36	314	154	391	+
	44	42	346	200	458	+
	47	50	472	142	177	+
	51	50	498	142	199	+
	52	50	390	124†	344	+

* — absent + present

† Above 160 mmHg at 2 wk after operation.

pear, in most cases, in the vessel walls proximal to the periphery of the heart. Our results show that by 16 weeks, or possibly earlier, renal hypertensive rats, when placed on an all-meat diet—high in fat and protein, but low in cholesterol—develop elevated serum cholesterol and coronary atherosclerosis. Whether the hypercholesterolemia precedes

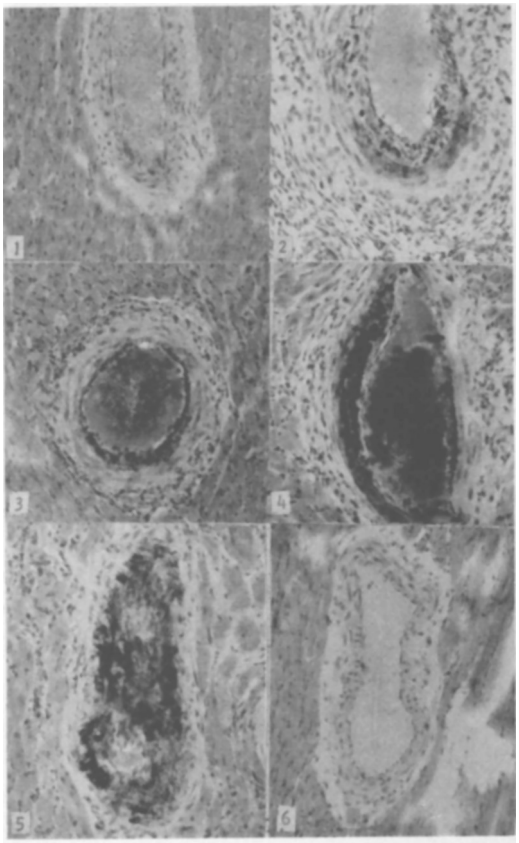

FIG. 1. Normal coronary artery from intact rat #75, 16 wk on meat diet, shows no evidence of vascular damage.

FIG. 2. Coronary artery from operated hypertensive rat #35, 16 wk on meat diet, shows massive perivascular inflammatory cell infiltration. Vessel wall has a thickened intima and medial fatty infiltration—early atherosclerosis.

FIG. 3. Coronary artery of operated rat #41, 26 wk on meat diet, has incipient inflammatory cell infiltration, slight thickening of intima with both subintimal and intramural lipid deposition—+1 atherosclerosis.

FIG. 4. Coronary artery from operated rat #46, 36 wk on meat diet, has an incipient inflammatory cell infiltration with massive fatty deposition in the media and intima with periadventitial and intramural fibrosis about the vessels—+2 atherosclerosis.

FIG. 5. Tangential cut of a coronary artery from operated rat #44, 42 wk on meat diet, shows massive subintimal and intramural lipid deposition —+3 atherosclerosis.

FIG. 6. Coronary artery from normal rat #74 shows no evidence of damage after 50 wk on meat diet.

and causes development of coronary atherosclerosis or is coincident with it and not an etiologic agent has not been clarified. This is being investigated.

Summary. Renal hypertensive rats on an all-meat diet developed hypercholesterolemia and coronary atherosclerosis within 16 weeks. Intact meat-fed, intact chow-fed, and renal hypertensive chow-fed rats developed neither condition by 50, 20 and 20 weeks respectively. Aortas of all animals chow- or meat-fed were normal. Thus, it appears that hypertension coupled with a high fat, high protein, but low cholesterol, diet is related to the production of hypercholesterolemia and coronary atherosclerosis. It is suggested that such a system may serve as an *in vivo* tool for the study of prevention, development and possible reversal of coronary atherosclerosis.

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Further Evidence for Reabsorption of Hemoglobin Iron Lost into the Intestine in Hookworm Infected Subjects.* (27425)

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Roche *et al.*(1,2) Foy and Kondi(3) Tasker(4) and Layrisse *et al.*(5) showed that considerable blood may be lost into the intestine by patients infected with hookworm. By means of a method involving the tagging of circulating erythrocytes with both Fe 59 and Cr 51(6), Roche and Pérez-Giménez(7) and Layrisse *et al.*(5) were able to measure intestinal iron loss as distinguished from fecal iron loss. It was found that fecal loss was on an average 55.9(7) and 64.10%(5) of intestinal loss, which would indicate that part of the hemoglobin iron was being reabsorbed. There was excellent correlation between fecal Fe 59 loss and reduction in blood Fe 59 activity, which would indicate that the absorbed iron was being utilized for fresh red cell synthesis (5).

Barun *et al.*(8) demonstrated, in hematologically normal patients injected with Fe 59, a progressive migration of radioactivity from the top to the lower levels of the packed red cell mass, as the erythrocytes aged with time. Eventually, radioactivity would again increase in the upper layer as the Fe 59 from the destroyed red cells was utilized for a new erythrocyte formation. Somewhat more efficient separation of erythrocytes of different age has been obtained by ultracentrifugation (9).

It was thought that, in cases with an abnormal iron loss into the intestine, as in hookworm infected subjects, followed by reabsorption and reutilization of such iron for hemoglobin synthesis, a constant supply of Fe 59 into freshly formed erythrocytes should result in radioactivity in the top layer of the hematocrit remaining at a relatively high level. If proved unequivocally to be true, this finding would provide further proof for iron reabsorption and reutilization from the intestinal tract.

Methods. Four heavily infected patients, with more than 5000 hookworm ova per gram of feces, and 3 normal non-infected subjects were selected for study. The patients were hospitalized in the wards of the Hospital Universitario in Caracas throughout the study

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