treatments are relatively non-toxic. There is a transient hypertrophy of the kidneys during the first few weeks of treatment with methionine but as prolonged treatment did not cause adrenal hypertrophy, it is doubtful that any of the regimens placed a severe stress on the animals.

Summary. The effects of prolonged administration of excess dietary dl-methionine and niacinamide on growth rate and on the relative weights of the liver, kidneys and adrenal glands of rats were determined. Although both methionine and niacinamide caused a decrease in the growth rate, a greater retardation of growth was obtained when both nutrients were administered together. The addition of 1% malic acid to the diet prevented the inhibition of growth by these substances. Although methionine caused a transient enlargement of the kidneys, none of the treatments appeared to place a severe stress on the animals.

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Prevention of Aortic Atherosclerosis in Rabbit by Intravenous Microcrystallized Estradiol Benzoate and Dextran®. (23770)

M. R. MALINOW, AMANDA A. PELLEGRINO, AND EUGENIA H. RAMOS
(Introduced by Chandler McC. Brooks)
*Pabellón de Cardiologia Luis H. Inchauspe, Hospital Ramos Mejia, Buenos Aires, Argentina**

It has been demonstrated that estrogens are able to prevent development of atherosclerosis in several species(1-3) and exert a beneficial effect even in man (see review in 4). Experiments designed to reproduce these results in male rabbits, however, have been consistently negative (personal communication) although contradictory conclusions have been reported in work done with female rabbits(5,6). In the present paper prevention of aortic atherosclerosis in cholesterol-fed male rabbits by a new method, *i.e.*, by the intravenous injection of a water-insoluble estrogen, is reported.

Methods. In this study male rabbits of mixed breed weighing 1.5 to 2.5 kg were maintained on carrots, clover, water and additional vitamins. All individuals, except the controls, also received 6 days a week 750 mg of crystalline cholesterol in 5 ml of sunflower seed oil (I. N. 130) thoroughly mixed with mashed carrots. One week after starting the cholesterol feeding, half of the animals thus fed were injected intravenously twice a week with 4 ml of saline in 2 divided doses, 2 hours apart; the other half received intravenously twice a week 2 ml of 6% Dextran® and 0.5 mg of microcrystallized estradiol benzoate† in 5 ml of saline, also in divided doses given 2 hours apart. Microcrystals less than 1 μ in diameter and suitable for intravenous

TABLE I. Aortic Macroscopic Atherosclerosis in Male Rabbits.

Group	No. of rabbits	Lesion grade				
		0	1	2	3	4
Control	12	12	0	0	0	0
Chol-saline	21	6	4	4	6	1
Chol-dext-estrad	22	14	1	5	2	0

Chol, cholesterol; dext, Dextran®; estrad, estradiol benzoate.

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† Estradiol-3-benzoate (Schering).

TABLE II. Percentage Distribution of Aortic Atherosclerosis in Cholesterol-Fed Rabbits.

Group	No. of rabbits	Without lesions	%	With lesions	%	"t"	p
Chol-saline	21	6	28.6	15	71.4	2.602	<.02
Chol-dext-estrad	22	14	63.6	8	63.3		

"t," Student's "t" test(8); p, probability; chol, cholesterol; dext, Dextran®; estrad, estradiol benzoate.

injection were freshly prepared by precipitating a concentrated alcoholic solution of estradiol benzoate into normal saline.

Phagocytosis of colloidal thorium has been demonstrated to occur in the atheromatous aorta of rabbits(8) and it is reasonable to suppose that the hormone microcrystals are phagocytized in the same way. It was felt that an even greater phagocytosis by blood vessel intima might be attained if uptake by the spleen were blocked. Therefore Dextran, a macromolecular polymer known to accumulate in the spleen of rabbits(9) was previously injected. Dextran itself has no effect on the development of atherosclerosis in cholesterol fed rabbits (unpublished data).

Results. The controls and 43 animals which survived 60 days of this experimental regime were sacrificed and their aortas examined. The observed macroscopic atherosclerosis was graded on an arbitrary 0 to 4 plus scale. Results shown in Table I demonstrate that the Dextran-estrogen-treated group had considerably less atherosclerosis than the saline-treated group. When the animals were rearranged as positive or negative for atherosclerosis and the conclusions assayed with Student's "t" test for the significance of percentages(7), the results were found to be statistically significant at the level of $p<0.02$ (Table II). Weight of the testicles and of the hypophyses showed no statistically significant differences in the three groups of rabbits (Table III).

Discussion. Although reasons accounting for prevention of atherosclerosis in the present experiment have not yet been explored, it is interesting to speculate concerning mechanisms probably involved, especially since similar experiments carried out even with higher doses of estrogens have been thus far consistently negative(5). It is possible that in our experiments, the hormone was more effective either because higher blood levels were obtained and/or because circulating microcrystals were phagocized by the altered endothelium, thus becoming veritable "intraarterial pellets."

The effects of microcrystallized estradiol in other arterial territories is under study at present; the application in man of this new method of administering drugs with a possible predominant action on altered arterial intima has already been tried in twenty patients without untoward results(10).

Summary. Intravenous administration of microcrystals of estradiol benzoate 2 hours after an intravenous injection of Dextran® prevented aortic atherosclerosis in cholesterol-fed rabbits. The testicle and the hypophysis weights were similar to control groups. A predominant action of the injected drugs in the arterial lesions is suggested.

TABLE III. Weight of Endocrines in Cholesterol-Fed Rabbits.

Group	No. of rabbits	Testicles (g)	No. of rabbits	Hypophysis (mg)
(1) Control	11	4.582 ± 1.678*	9	21.51 ± 5.15
(2) Chol-saline	20	4.227 ± 1.805	15	22.8 ± 7.24
(3) Chol-dext-estrad	21	3.986 ± 1.152	16	21.3 ± 7.65
"t" 1 *vs* 2		.538 (p <.6)		.522 (p <.6)
" 1 *vs* 3		1.185 (p <.3)		.073 (p >.9)
" 2 *vs* 3		.507 (p <.7)		.177 (p <.9)

* Stand. dev.

Chol, cholesterol; dext, Dextran®; estrad, estradiol benzoate; "t," Student's "t" test; p, probability.

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Effect of Nephrosis on Renal Q_{O_2} and Histochemical Appearance of Cytochrome Oxidase and Succinic Dehydrogenase. (23771)

EDWIN R. FISHER, F. I. TSUJI AND J. GRUHN

Departments of Pathology, University of Pittsburgh and Veterans Admin. Hospital, Pittsburgh, Pa.

Investigation of the appearance and distribution of certain histochemically demonstrable renal tubular enzymes in rats with nephrosis induced by injection of anti-rat kidney-rabbit serum revealed an appreciable depletion of cytochrome oxidase (G-nadi oxidase) and succinic dehydrogenase(1). Certain physiologic and morphologic considerations suggested that these alterations were related to degree of proteinuria rather than to a nephrotoxic effect of the serum employed. It therefore appeared worthwhile to evaluate such enzymatic activity in nephrosis produced by another etiologic agent and to attempt to quantitate any changes if present, manometrically. In addition, this information would also provide an opportunity for correlation of results obtained by the *in vitro* and histochemical staining technics. In the present study nephrosis was induced by appropriate doses of aminonucleoside, an agent which has been demonstrated to produce such a state with relative ease and a high degree of consistency(2,3).

Methods. Forty-two adult female Wistar rats weighing 150-200 g were utilized. Group A consisted of 21 animals that received 0.003 ml of a 0.5% aqueous solution of aminonucleoside* per g of body weight subcutaneously for 9 consecutive days and were sacrificed 5 days after the last injection. There were 8 animals in Group B that received a similar dose of aminonucleoside but were sacrificed after only 6 injections. Group C was comprised of 13 untreated rats of similar sex, strain and weight. Protein content of the urine was determined daily and the serum cholesterol, total lipid and total protein at the time of sacrifice by methods described elsewhere(1). All animals were sacrificed by decapitation. Their kidneys were quickly removed. One was placed immediately in ice after removing its capsule and ureter. Thin slices consisting of the cortex and medulla were prepared according to the method of Deutsch(4). These were incubated in Krebs-Ringer-phosphate-glucose buffer, pH 7.2 at 37 C in a total volume of 3.0 ml. The gas phase consisted of 5% CO_2 and 95% O_2. Liberated CO_2 was absorbed by 20% KOH placed in the center well. The oxygen consumption was followed from 30 to 60 minutes, after which time the tissues were removed and dried at 108°C for 2 hours. Their dry weights were obtained after desiccation for 18 hours. 0.6 ml of a 0.5% solution of aminonucleoside, previously neutralized and

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