Experimental Arteriosclerosis: Inhibitory Effects of Ascorbic Acid and Inositol. (19850)

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The term arteriosclerosis has been variously defined. Too commonly, lipid, and in particular cholesterol infiltration (atheroma), has been identified as the essential lesion in this syndrome. For purposes of this paper we have defined arteriosclerosis as any thickening, or increase in density, of the arterial wall. Atherosclerosis may be defined as a degenerative metabolic subdivision of arteriosclerosis, characterized by hyaline degeneration, lipid infiltration and finally, calcinotic changes of the intima and/or media.

Our immediate purpose in investigating this problem, was 2-fold: 1. To produce experimentally, in the rabbit, arteriosclerotic lesions of the aorta. 2. To inhibit or reverse the formation of these lesions. Josue(1) observed experimentally, that repeated injections of epinephrine were capable of producing sclerosis of the aorta. Numerous investigators(2,3) have, since that time, reaffirmed this experimental fact, usually describing the resultant plaques as erosions or necroses of the tunica media. Oester and Mikulicich(4) have recently observed that combined injections of epinephrine and thyroxine result in an apparent potentiation in the production of this experimental aortic sclerosis. The present communication is a report on the results of experiments with inositol and ascorbic acid, used separately, in an endeavor to influence the epinephrine-thyroxine induced arteriosclerosis in rabbits.

Methods. Rabbits totalling 111, were used. The method for production of the sclerosis was as follows. Twenty-five μg of epinephrine hydrochloride per kilo of body weight were very slowly injected intravenously by ear, on 2 consectutive days. On the third day the dose of epinephrine injected was increased to 40 μg per kilo. On the fourth day it was increased to 50 μg per kilo. This regimen of 50 μg of epinephrine per kilo of body weight was maintained for 11 more days, a total of 15 days of epinephrine administration. Thyroxine was injected subcutaneously, in the region of the middle of the back, daily, for the 15 days, at a constant dose level of 0.15 mg/kg body weight. Post-mortem examinations were routinely made. Our primary observations were concerned with the frequency and severity of sclerosis in the aorta from the region of the heart into the iliac bifurcation. Final conclusion as to whether sclerosis was actually present was based on histological study. It is virtually impossible to compare plaques accurately, but for purposes of differentiation we undertook their gradation on the following basis: zero, no plaques; 1, mild single patches (plaques); 2, mild, scattered patches; 3, severe, extensive patches; 4, widespread, confluent and extensive patches. The control group of 25 animals received only epinephrine and thyroxine injections. Identical regimens of epinephrine and thyroxine injections were also administered to the groups of animals which received inositol, and to those which received ascorbic acid. Thirty-six animals were treated with ascorbic acid, 18 received 100 mg per day, 9 received 500 mg per day, and 9 received 50 mg per day. All ascorbic acid was administered subcutaneously in the region of the middle of the back. The ascorbic acid treatment was begun 3 days before epinephrine and thyroxine. All injections were continued for 15 more days, thereby totalling 18. Thirty animals were treated with inositol, the subcutaneous injections being given, as with Vit. C, before and during the epinephrine and thyroxine for a total 18 day program. The animals were divided by dose of inositol into 2 groups of 15 each, the first receiving 3 g per kilo per day, the second 6 g per kilo per day. Table I provides a summary of the protocol from these experiments. Table II is an analysis of the data from Table I.

Results and discussion. Investigation with massive doses of inositol indicates it has no significant value in the treatment or prophylaxis of the experimental epinephrine-

TABLE I. Influence of Inositol and Ascorbic Acid on Epinephrine-Thyroxine Sclerosis.

Treatment*	No. of rabbits	Deaths before 6 days	Deaths 6 to 15 days	No. animals sacrificed on 16th day	Degree of sclerosis 0	1	2	3	4
E + T (C)	25	5	12	8	2	4	4	3	7
C + I, 3 g/kg	17	2	12	3	3	5	3	3	1
" 6 "	19	4	11	4	4	4	3	4	0
C + A, 50 mg/kg	12	3	5	4	3	2	1	1	2
" 100 "	22	4	11	7	7	2	4	3	2
" 500 "	10	1	6	3	4	1	2	2	0

* E = Epinephrine; T = Thyroxine; C = Controls; I = Inositol; A = Ascorbic acid.

TABLE II.
Significance of Influence of Inositol and Ascorbic Acid on Epinephrine-Thyroxine Sclerosis.

	No. with sclerosis /Total No. animals	% incidence	Probability*	Severity factor (% incidence × avg degree of sclerosis)
E + T (C)†	18/20	90		90 × 2.45 = 220
C + I, 3 g/kg	12/15	80	>.1	80 × 1.6 = 128
" 6 "	11/15	73	>.1	73 × 1.46 = 106.5
C + A, 50 mg/kg	6/9	66	>.2	66 × 1.66 = 109.5
" 100 "	11/18	61	<.01	61 × 1.5 = 91.5
" 500 "	5/9	55	>.1	55 × 1.22 = 67.1

* Probability compared to controls, a value of <.05 is assumed significant. Probability: (1) for total inositol group = >.1; (2) for total ascorbic acid group = <.01. Animals whose death occurred before 6th day of treatment were not included in biometrical analysis.
† E = Epinephrine; T = Thyroxine; C = Controls; I = Inositol; A = Ascorbic acid.

thyroxine arteriosclerosis. Ascorbic acid proved valuable in the inhibition of these lesions, especially in daily doses of 100 and 500 mg. The tables indicate the results which were obtained. The data obtained with the use of inositol, although it demonstrates a lower incidence of sclerosis, is not of statistical significance. The results with ascorbic acid are of statistical significance. In addition, the severity of the sclerosis developed under the influence of ascorbic acid was markedly less than either the controls or when inositol was used. This is shown by the lessened number of cases which were recorded as four plus sclerosis. In addition, a factor which we call severity factor also expresses this relationship. This number is obtained by multiplying the percentage incidence of sclerosis in any group by the average degree of sclerosis, expressed in our plus units. The higher this number, the higher is the total gross degree of sclerotic severity. It is seen that this value is markedly lower with each of the ascorbic acid groups and there is a progressive decrease with increasing dose of ascorbic acid.

The severe median and often intimal sclerosis induced in the aorta of rabbits by combined injections of epinephrine and thyroxine indicates the possibility that such a combination of hormonal influences may be active in the production of arteriosclerosis in other animals.

Epinephrine scleroisis, potentiated by thyroxine and perhaps by other hormonal imbalances, may exert sufficient injury to the arterial wall so that later, lipid deposition is favored. The experimentally demonstrable influence of epinephrine and thyroxine might be considered as part of the pre-lipid phase of atherosclerosis. It is our feeling that some shock phase precedes active lipid-infiltration. We suggest that a dysfunction of lipid metabolism may be a factor which is secondary to the lesions resulting from these shocking stresses, hormonal or otherwise.

The role of thyroxine in the production of arteriosclerosis is paradoxical and, as yet, not well defined. It has been used successfully to produce remission of the experimental cholesterol sclerosis(5) and yet as stated it adds significantly to the degree and incidence of the sclerosis produced by epinephrine in the rabbit. This last fact is supported somewhat

by the findings of Lortat and Sabareaunu(6) who inhibited the epinephrine-induced sclerosis by extirpation of the thyroid gland.

Summary. 1. Combined injections of epinephrine and thyroxine were used to produce, in rabbits, aortic sclerosis which was characterized by lack of atheroma, and the presence of severe medial and intimal sclerosis. 2. 90% of all the control animals developed aortic sclerosis when treated with epinephrine and thyroxine. 3. Massive doses of inositol appeared to inhibit the influence and severity of these lesions, to only a slight degree. 4. Daily concomitant administration of 50 mg of ascorbic acid lowered the incidence of scleroses to 66%; 100 mg lowered the incidence to 61%; 500 mg lowered it to 55%. The severity of the lesions produced was also considerably lessened. The inhibition due to ascorbic acid was statistically significant.

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Effect of Bacteria and Their Products on Migration of Leukocytes.* (19851)

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In the course of studies of a purified toxic somatic antigen from *Salmonella typhosa*, Morgan(1) noted that leucocytes from guinea pig splenic explants failed to migrate towards a capillary tube containing this material. The technic used in this test was that of Chambers and Legrand(2) which consisted of placing the bacterial product in a capillary tube adjacent to the explant and noting the direction of migration of the leucocytes. The failure to migrate toward the typhoid somatic carbohydrate was interpreted as negative chemotaxis. When whole typhoid bacilli were employed, slight positive chemotaxis was noted. It was reported by Delauney and Pages(3) that the injection of the "endotoxins" of *Salmonella typhosa* prevented the diapedesis of leucocytes from the blood vessels of animals. Miles and Nivens(4) confirmed these findings and believed that this occurred because of poor tissue perfusion secondary to the circulatory failure produced by the endotoxins. The present study deals with the effect of a series of bacteria and some bacterial products on leucocytes. The tests were carried out in a special cell(5) which permits quantitation of the capacity of leucocytes to migrate from a buffy coat culture.

Methods and material. Human blood was used, silicone (GE No. 9986) treated glassware, syringes, and needles were employed throughout; heparin in final dilution of 1 μg per ml of blood was used to delay clotting. The organisms or purified bacterial products were added to the blood in quantities outlined in the tables. The bacterial products were suspended in isotonic saline and the final dilution of one part of the material to 9 parts of blood was used. The slide cell for centrifugation has been modified by the use of 2 lucite blocks held together by screws and separated by a rubber band (Fig. 1). Cells were filled to the midway mark with blood and the bolts

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