EFFECTS OF ONION IN ATHEROSCLEROSIS IN RABBITS. III. REDUCTION OF NUCLEIC ACIDS AND PROTEINS IN AORTA

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ABSTRACT

The effects of administration of atherogenic diet and onion extract on the levels of nucleic acids, total protein, collagen and elastin were studied in albino rabbits. The levels of these constituents are higher in the cholesterol-fed animals than in the cholesterol plus onion extract fed animals, at comparable plasma cholesterol levels. The levels in the latter group are slightly higher than in the normal control group. These findings suggest that the feeding of onion extract helps to maintain these parameters close to normal levels in atherosclerotic rabbits.

INTRODUCTION

RECENTLY we have reported the regressive effects of onion extract on the erythrocytes and the aortic lesions in rabbits. In animals on cholesterol enriched diet, the erythrocytes tend to crenate and aggregate, whereas, in the animals fed the same diet and onion extract, the erythrocytes have normal characteristics[1,2]. In the aortic vessel of the latter, the lesions are significantly less compared to cholesterol-fed animals at the end of six months[3].

The regressive effect of onion extract on aortic tissue cholesterol level is comparable with the various aortic cholesterol lowering agents while the serum cholesterol was maintained at high levels[4–6].

It has been reported that onion extract increases the fibrinolytic activity and decreases slightly the plasma cholesterol and fibrinogen levels in hyperlipidemic animals and human beings[7–10] but to authors' knowledge no attempts have been made to study the effects of this extract on the levels of nucleic acids and proteins in the aortic tissues. In this paper the effect of onion extract on the levels of nucleic acids and proteins, in the animals fed on atherogenic diet is reported and is compared with the levels of normal control animals.

MATERIALS AND METHODS

White albino male rabbits of same age (2-3 months) and body weight were initially fed with a normal diet (carrot, cabbage and greens). The animals were then divided into three groups (10 animals in each group). Group I served as normal controls, Group II animals were fed an atherogenic diet (normal diet plus 0.5% cholesterol), and Group III animals were given the same atherogenic diet with the addition of extract of 20 g of fresh onion. The onion extract was water soluble and was prepared by the method of Stoll and Seebeck[11]. All the animals consumed the same amount of diet and the weight gain followed a similar pattern. The plasma cholesterol levels in Groups II and III were maintained between 1000–1400 mg% by the dietary adjustment of cholesterol intake. At the end of six months the animals were killed. Blood was drawn by heart puncture before autopsy. At autopsy, the entire aorta was removed for the study. Portions of ascending aorta, taken for histopathological studies, were fixed in 10% formalin and the remainder was used for lipid, protein and nucleic acid estimations.

The total lipids from plasma were extracted by the method of Leffler[12] and from the aorta by the method of Folsch *et al*[13]. The blood and tissue cholesterol were measured by the method of Abell *et al*[14]. Phospholipids were determined by analysis of the phosphorus content by Fiske and Subbarow method[15] and multiplying by a factor of 25. The aortic DNA, RNA and total protein were measured by Burton[16], Schneider[17] and Lowry[18] methods, respectively. Elastin and collagen were extracted[19] and estimated by the method of Newman and Logan[20].

RESULTS

The total plasma lipids, cholesterol and phospholipids levels of three groups of rabbits are given in table 1. The total lipids in Group III has increased by 3.5 times and in Group II by 5 times, when compared to Group I. The total cholesterol for Groups II and III is comparable. The level of phospholipids is highest in Group III.

The levels of lipids (total lipids cholesterol and phospholipids), nucleic acids (DNA and RNA), total protein, elastin and collagen of aorta, for various groups of animals, are shown in table 2. The total

Table 1 *Plasma Lipids*

Group	Total lipids (mg %)	Cholesterol (mg %)	Phospholipids (mg %)
I	1000 ± 125[a]	142 ± 32	136 ± 19
II	5308 ± 280[b]	1228 ± 360[b]	249 ± 24[b]
III	3598 ± 450[b]	1148 ± 310[b]	409 ± 29[b]

[a] Mean ± SD; [b] *p* values: < 0.005 *to* < 0 0005

lipids of tissues of Group III were slightly increased, whereas, in the Group II this increase is highly significant when compared to Group I. The cholesterol, phospholipids, nucleic acids, collagen and elastin levels for Group III were decreased by 50% compared to Group II but the levels were higher than Group I.

The microphotographs of the paraffin sections of the aorta are shown in figures 1, 2 and 3. No intimal lesions were observed in the control animals (figure 1). Group II animals (figure 2) had prominent intimal lesions, comprised principally of smooth raised or elevated lipid and fibrolipid plaques on the posterior wall of the aorta involving one half of the intimal surface (Grade II by W.H.O. Standards).

Group III animals showed small, very thin and slightly elevated lipid deposits on the posterior wall with intervening normal intima involving less than 10% of the intimal surface (figure 3) (Grade I by W.H.O. Standards).

DISCUSSION

The results presented here show that the induction of atherosclerotic lesions in rabbits by dietary supplementation of cholesterol is characterized by stimulation of nucleic acids and protein synthesis in the aortic tissues. The results indicate that the observed increase in collagen is just one of the several changes occurring with respect to protein metabolism in atherosclerotic rabbits. These results suggest that the increased protein synthesis in aorta in Group II animals may be a reflection of the increased number of cells in the thickened intima. The cells, which presumably are in the active phase of proliferation and growth at least, in part, are responsible for the increase in protein synthesis. Similar increase in protein synthesis, especially collagen, has been observed in atherosclerotic rabbits and pigeons[21-26].

The reduction of collagen and elastin levels to almost normal levels in Group III rabbits, which were maintained at high plasma cholesterol levels (as in Group II) but were simultaneously fed onion extract, show that the onion extract in some way helps to regress atherosclerosis. A comparison of proteins and nucleic acid levels between the various groups studied indicates that these levels have been only slightly increased in Group III, whereas, they have considerably increased in Group II (compared to controls). The fraction of the total protein (TP) synthesized accounted for by collagen (C) and elastin (E) for various groups are given in table 3.

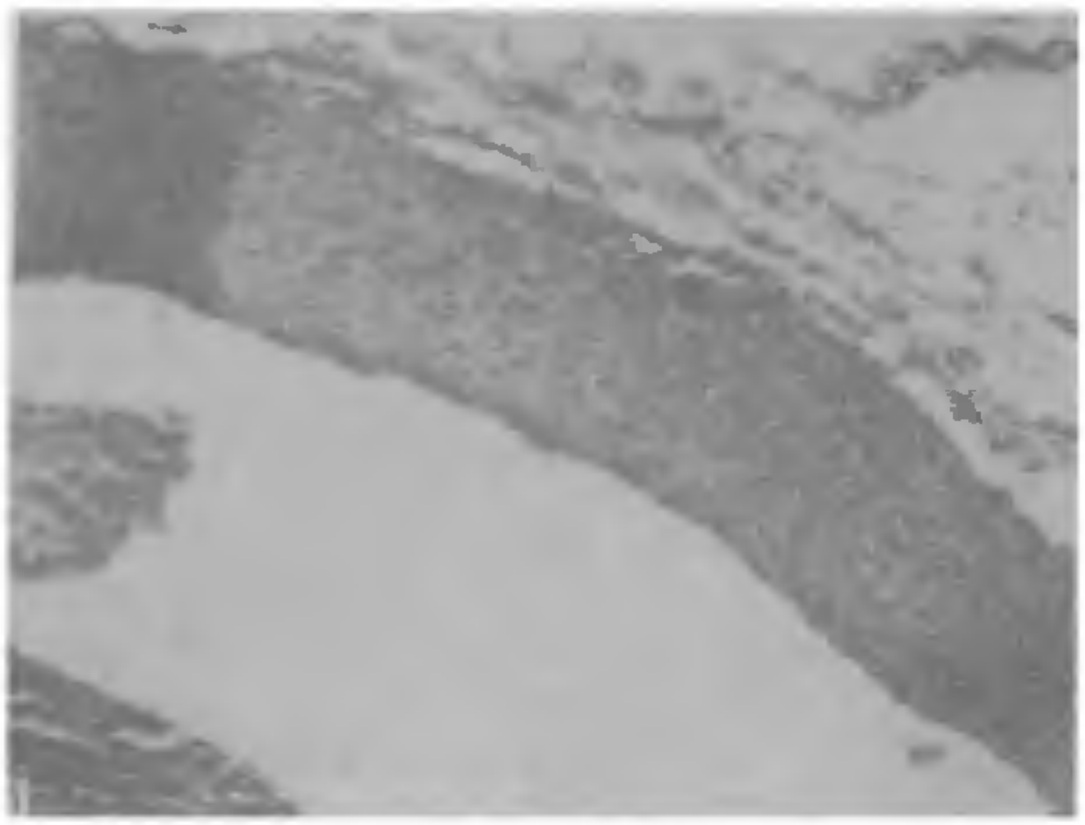
Increased C/TP and E/TP values suggest that the collagen and elastin synthesis is enhanced during the atherosclerotic process. This increase in Group II is significantly higher than that of normal controls which is in agreement with the observation of others[27-29]. A comparative study between Groups II and III shows that the administration of onion extract has significantly affected the synthesis of collagen and elastin in the aortic tissues which is further substantiated by the formation of lesions of various grades in the blood vessels.

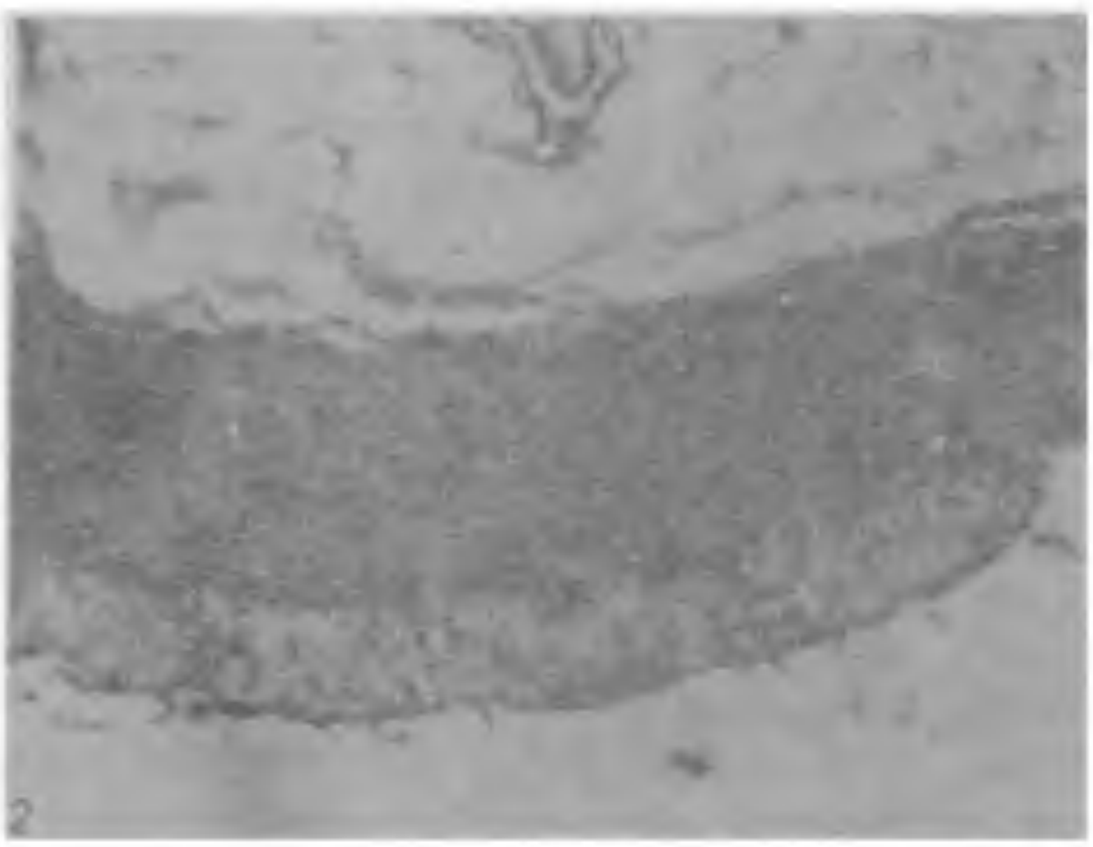
The increase of nucleic acids in Group II is significantly higher than that of other groups which is in conformity with an earlier report[30]. This enhancement may be attributed to the alteration in the lysosomes of

Table 2 *Aortic Constituents*

Tissue constituents mg/g dry tissue	Group I	Group II	Group III
Total Lipids	80 5 ± 10 9[a]	150.0 ± 19.2[b]	95.8 ± 20 9[c]
Cholesterol	3.8 ± 0.5	35 6 ± 8 4[b]	13.2 ± 3 3[b]
Phospholipids	3 6 ± 0.4	24 2 ± 8.7[b]	13.7 ± 4 3[b]
DNA	6 6 ± 0.3	20 0 ± 1 2[b]	9 3 ± 0 7[b]
RNA	0.8 ± 0.1	4 0 ± 0 1[b]	1 0 ± 0 1[c]
Total Protein	80 5 ± 3 4	152 2 ± 10 2[b]	93 6 ± 4 7[b]
Elastin	4.2 ± 0.5	12 1 ± 1 1[b]	6 5 ± 0 8[b]
Collagen	6 2 ± 0 5	19 9 ± 2 1[b]	9 2 ± 1 1[b]

[a] Mean ± S D; [b] *p*-values. < 0 005 to < 0 0005, [c] *p*-values < 0 05 to < 0 01





Figures 1–3. 1. Aorta of normal rabbit. H–E, 2. Aorta of Group II rabbits with lipid rich lesions, and 3. Aorta of Group III rabbit showing smaller internal lesions, (× 100).

arterial smooth muscle[31] which is significantly higher in group II as compared to others[32].

Table 3 *Elastin and collagen to total protein ratios*

Groups	E/TP	C/TP
I	0 053 ± 0.003[a]	0.077 ± 0 001
II	0.079 ± 0.003[b]	0.131 ± 0 002[b]
III	0 069 ± 0 005[b]	0.099 ± 0 002[b]

[a]mean ± S D.; [b]*p*-values: < 0 005 to < 0.0005

These findings suggest that the administration of onion extract along with atherogenic diet leads to the reduction of the deleterious effect of hypercholesterolemia on the structural constitutents of blood vessels. This mechanism may directly be associated with erythrocytes (as the cells maintain their normal shape and deformability[33] which maintain the normal supply of oxygen to tissues) and indirectly in the form of providing a protective layer at the endothelium which may retard the cholesterol accumulation in the blood vessels. Further studies are in progress to understand the mode of action of onion.

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NEWS

"REGISTRATION CERTIFICATES" FOR STARS

It has taken astronomers just a few hours to fill the registration certificate where the size, weight, temperature and other parameters of Arcturus—a star from the Bootes constellation—are recorded.

Previously such work took about six months. This progress has been made due to a special deciphering set, created by scientists of the astronomical observatory of Odessa University.

"The new complex has united a powerful telescope, a measuring device and a mini-computer," said Yu. Medvedev, Cand. Sc. (Phys.-Math.), chief of the observatory. "At first, the light signals of far-off stars get through the telescope into the measuring device—the spectrophotometer. The latter transforms these signals into electric pulses and then sends them to the computer. Performing numerous mathematical operations, the computer yields exact data on the energy of the star, the chemical composition of the atmosphere, the temperature, gas pressure and dozens of other parameters," the scientist noted. (*Soviet Features*, Vol. XXIII, No. 176, November 22, 1984).