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Effects of Some Vasoactive Agents on Isolated Blood Vessels from Cholesterol-fed Rabbits

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The effects of several vasoactive agents on the blood vessels from cholesterol-fed rabbits were investigated. Fourteen rabbits fed with cholesterol-containing pellets (2% cholesterol and 6% corn oil) showed an increase in a total blood cholesterol level up to 1142 ± 98 mg/dl in three months. Various macroscopic changes in the blood vessels and other tissues of cholesterol-fed rabbits were observed at the end of three-month feeding, *e.g.*, fat deposition in the subcutaneous tissue of ear and iris, atheromatous plaques in the intima of thoracic aortae and fatty livers. In the isolated hearts, there was no significant difference between cholesterol-fed and control animals in coronary vasodilatation caused by glyceryl trinitrate, 5 μg/ml, and papaverine, 10 μg/ml. The perfused central ear arteries from cholesterol-fed rabbits showed a tendency to be less sensitive to norepinephrine, $10^{-9}-10^{-5}$, histamine, $10^{-9}-3\times10^{-5}$ and KCl, $3\times10^{-3}-3\times10^{-1}$ g, than those from control animals. In aortic strips from cholesterol-fed rabbits, norepinephrine, $3\times10^{-9}-3\times10^{-5}$, angiotensin, $10^{-9}-3\times10^{-6}$ and $BaCl_2$, $3\times10^{-5}-10^{-2}$ g/ml, caused greater contractions than in the controls. On the contrary, histamine, $10^{-8}-3\times10^{-4}$, and KCl, $7.5\times10^{-4}-2.2\times10^{-2}$ g/ml, induced smaller contractions in the atherosclerotic aortae than in the controls. The descending aortae were generally more sensitive to the agents than the ascending aortae. Such various changes suggest that the atherosclerotic development in the blood vessels influenced either favorably or unfavorably on the contraction of the smooth muscle depending on the agents, although its detailed mechanisms need further investigations.

The fact that the properties of vascular tissues are influenced by pathological states, including atherosclerosis, has been widely known. Various morphological and histochemical changes observed in patients with atherosclerosis and in experimentally induced atherosclerotic animals have been reviewed.[2] Although electrocardiographic studies on atherosclerotic animals under hypoxia have been reported,[3] little attention has been paid on the resultant pharmacodynamic changes in the vasculature in atherosclerotic animals.

In the present study, an attempt was made to determine the response of the blood vessels isolated from cholesterol-fed rabbits to several vasoactive agnets.

Experimental

Methods——1. Cholesterol Feeding and Blood Cholesterol Level Determination: Fourteen male albino rabbits weighing about 2.5 kg were fed with rabbit pellet diet (Nihon Clea, CR-1) containing 2%

1) Location: *Hongo, Bunkyo-ku, Tokyo.*

2) A.I. Lansing (ed.), "The Arterial Wall: Aging, Structure and Chemistry," The Williams & Wilkins Co., Baltimore, 1959; D.I. Abramson (ed.), "Blood Vessels and Lymphatics," Academic Press, Inc., New York, 1962; R.J. Jones (ed.), "Evolution of the Atherosclerotic Plaque," The University of Chicago Press, Chicago, 1963; J. Orbison and E. Smith (ed.), "The Peripheral Blood Vessels," The Williams & Wilkins Co., Baltimore, 1963; M. Sandler and G.H. Bourne (ed.), "Atherosclerosis and Its Origin," Academic Press, Inc., New York, 1963; J.C. Roberts, Jr. and R. Straus (ed.), "Comparative Atheorsclerosis," Harper & Row Publishers, New York, 1965; R. Paoletti (ed.), "Experimental Atherosclerosis in Lipid Pharmacology," Academic Press, Inc., New York, 1964.

3) M.M. Winbury, J.K. Wolf, and M.T.I. Cronin, *Am. J. Physiol.*, **200**, 642 (1961); D.R. Varma and K. Melville, *Am. J. Cardiol.*, **9**, 471 (1962); I.I. A. Tabachnick, L. Szybalski, and M.M. Winbury, *Arch. int. Pharmacodyn.*, **154**, 395 (1965).

cholesterol and 6% corn oil for three months. Eleven rabbits fed with the diets without adulterants were used as a control group. Water was supplied *ad libitum* to both groups. A total blood cholesterol level was measured every two weeks by modified Zak-Henley method[4]: 8 ml of glacial acetic acid solution of ferric chloride (0.08%) was mixed with 0.1 ml of blood sample, kept at room temperature for 15 min and centrifuged. The supernatant, 6 ml, was vigorously mixed with 4 ml of sulfuric acid. Absorbance of the resultant purple color was measured at 560 mμ by a spectrophotometer (Hitachi 124).

2. Coronary Artery in Isolated Hearts: Rabbits were sacrificed by a blow on the head, and the heart was dissected immediately. The coronary artery was cannulated via the aorta, and perfused under constant pressure with Tyrode solution oxygenated with a mixture of 95% O_2 and 5% CO_2 at 37°, according to modified Langendorff technique.[5] The agents studied were added to the perfusion fluid in separated reservoirs. The heart was fibrillated by means of alternate current, 50 Hz, 4–12 volts, applied to the wall of the left ventricle close to the apex through a pair of platinum electrodes. The vasodilating effect of the agents was estimated by counting the number of drops flowed out during the period of 15 sec.

3. Isolated Central Ear Artery: The isolated central ear artery was perfused according to the methods of De La Lande, *et al.* and Steinsland, *et al.*[6] A 20 mm portion of the artery was isolated from the base of the ear, cannulated with polyethylene tube at both ends and mounted in a 20 ml organ bath filled with modified Krebs solution (NaCl 118.9, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 24.9, and dextrose 5.6 mM) saturated with a mixture of 95% O_2 and 5% CO_2 at 37°. The artery was simultaneously perfused intraluminally with a pulsatile pump (Sigma Motor, T–8) at the rate between 12 and 16 ml/min, which was associated with resulting level of perfusion pressure between 80 and 120 mmHg. The perfusion pressure of intraluminal inflow was measured with a pressure transducer (Nihon Kohden, MPU–0.5) and was recorded on a polygraph (Nihon Kohden, RM–150). Each drug solution was injected into the intraluminal perfusing fluid just before the artery. The doses were expressed as a total amount injected.

4. Aortic Strips: The ascending and descending thoracic aortae were cut spirally into strips of 2×30 mm. The strips were mounted under a resting tension of 1 g in a 10 ml organ bath containing Krebs bicarbanate solution bubbled with a mixture gas of 95% O_2 and 5% CO_2, and allowed to equilibrate for 90 min before drug application. Responses were recorded isotonically with 5-fold magnification on a smoked kymograph paper. Drug solution were administered according to the cumulative method of Van Rossum.[7]

Materials——The agents used were as follows: cholesterol (Tokyo Kasei Co.) as adulterant to feeding pellets, glyceryl trinitrate (Nihon Kayaku Co.), papaverine hydrochloride (Iwaki Seiyaku Co.), norepinephrine hydrochloride (Sankyo Co.), histamine dihydrochloride (Daiichi Pure Chemicals Co.), angiotensin II (Ciba Pharmaceuticals Co.), potassium chloride (Wako Pure Chemicals Co.), barium chloride (Wako Pure Chemicals Co.), glacial acetic acid for cholesterol determination (Daiichi Pure Chemicals Co.), conc. sulfuric acid (Wako Pure Chemicals Co.), and ferric chloride (Wako Pure Chemicals Co.). Agents used for pharmacological test were freshly dissolved in physiological saline into the required concentrations. The dose of the agents was expressed in terms of salt.

Results

1. Macroscopic Observations and Cholesterol Level

The total blood cholesterol level before the onset of cholesterol feeding was 346±32 mg/dl and 366±15 mg/dl in the control and cholesterol-fed rabbits, respectively. In the latter group, the value began to increase in the second week and continued to increase to the tenth week up to 1142±98 mg/dl. The value at the time of sacrifice (the twelfth week) was 960±165 mg/dl. In the control group the value kept minor fluctuation over a period of the feeding, as shown in Fig. 1. Body weight of the cholesterol-fed rabbits increased to the sixth week and gradually decreased thereafter.

Various macroscopic alterations in the blood vessels and other tissues of cholesterol-fed rabbits were observed at the end of three-month feeding. Fat was deposited in the subcutaneous tissues of ears and irises. Atheromatous plaques developed in the intima of thoracic aorta, especially in the ascending aorta. The descending thoracic aorta near the abdominal aorta showed a moderate atheromatous change, but some rabbits showed no atheromatous

4) B. Zak, *Am. Clin. Pathol.*, **27**, 583 (1957); R.G. Henley, *Analyst*, **28**, 286 (1957).
5) R. Charlier, "Coronary Vasodilators," Pergamon Press, Oxford, 1961.
6) I.S. De La Lande, V.A. Cannel, and J.G. Waterson, *Brit. J. Pharmacol.*, **28**, 255 (1966); O.S. Steinsland, R.F. Furchgott, and S.M. Kirpekar, *J. Pharmacol. Exptl. Therap.*, **184**, 346 (1973).
7) J.M. Van Rossum, *Arch. int. Pharmacodyn.*, **143**, 299 (1963).

plaque. In the central ear artery, atheroma was seldom seen even when thick fatty tissue deposition in the perivascular tissue was observed. The coronary artery showed no atheromatous change. Fatty livers were observed in all cholesterol-fed rabbits.

Three rabbits fed with cholesterol-containing pellets and one in the control group died during the period of feeding and were ommitted from the experimental data.

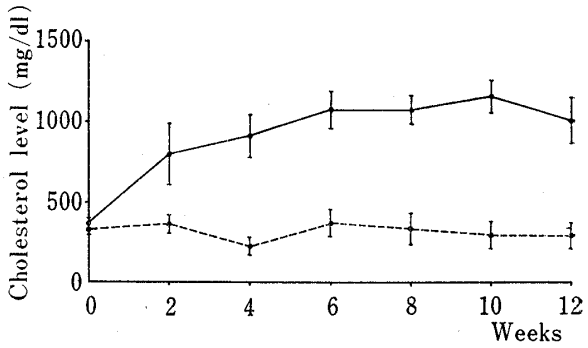

Fig. 1. Changes in Total Blood Cholesterol Levels of Cholesterol-fed and Control Rabbits

Abscissa: time in weeks after the onset of cholesterol feeding; ordinate: total blood cholesterol level in mg/dl. Solid line shows cholesterol-fed rabbits ($N=14$) and broken line control animals ($N=11$). Vertical bars represent standard errors of mean values.

2. Coronary Artery in Isolated Hearts

Glyceryl trinitrate, 5 μg/ml, and papaverine, 10 μg/ml, increased the average outflow by 13.4±2.4 ($N=6$) and 15.3±4.6 (S.E.M.) drops/15 sec ($N=6$), respectively, in the cholesterol-fed rabbit hearts. On the other hand, the same doses of glyceryl trinitrate and papaverine increased the average outflow by 10.6±1.6 ($N=9$) and 14.6±4.6 drops/15 sec ($N=7$), respectively, in the control rabbit hearts (Table I). However, no statistically significant difference between these two animal groups for each agent was observed. The hearts isolated from cholesterol-fed rabbits appeared to deteriorate faster than those from control animals.

TABLE I. Effects of Glyceryl Trinitrate and Papaverine on Coronary Outflow of Isolated Hearts from Cholesterol-fed and Control Rabbits

Agent		Increase in coronary outflow (drops/15 sec)	
		Cholesterol-fed rabbit heart	Control rabbit heart
Glyceryl trinitrate	5 μg/ml	13.4±2.4[a] (6)[b]	10.6±1.6 (9)
Papaverine	10 μg/ml	15.3±4.5 (6)	14.6±4.6 (7)

a) Values represent mean±standard error
b) Figures in parentheses show the number of experiments.

3. Isolated Central Ear Artery

Dose-response curves for norepinephrine, 10^{-9}—10^{-5} g, histamine, 10^{-9}—3×10^{-5} g, and potassium chloride, 3×10^{-3}—3×10^{-1}, are shown in Fig. 2. In each experiment, five preparations for cholesterol-fed rabbits and four for control animals were used. The central ear arteries isolated from cholesterol-fed rabbits showed a tendency to be less sensitive to those agents than those from control animals. Typical responses of the arteries to norepinephrine are illustrated in Fig. 3. The minimum doses of histamine and potassium chloride required to contract the preparations from cholesterol-fed rabbits were slightly greater than those in the controls. The response to potassium chloride was much less in the cholesterol-fed rabbit ear arteries than in the controls, although there was no statistically significant difference between the two groups. In two of five preparations, practically no response to the agents was observed.

4. Aortic Strips

Dose-response curves for norepinephrine, angiotensin, histamine, potassium chloride and barium chloride are shown in Fig. 4. In each curve, seven preparations for cholesterol-fed

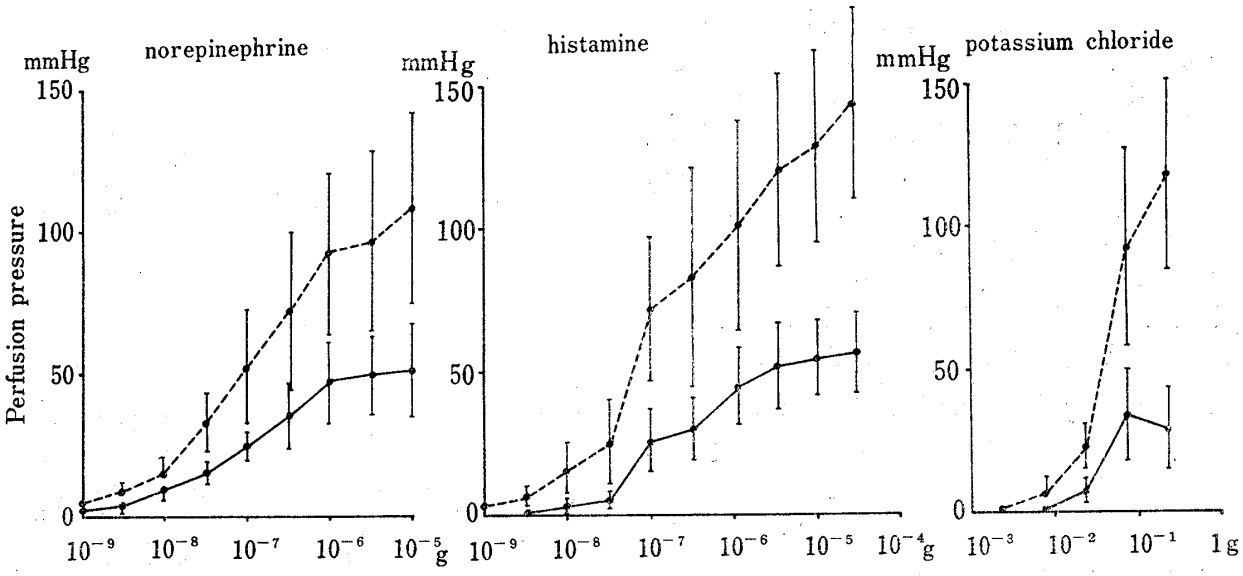

Fig. 2. Dose-response Curves for Norepinephrine, Histamine and Potassium Chloride in the Isolated Central Ear Arteries from Cholesterol-fed and Control Rabbits

Abscissa: doses of the agents in g as the total amount injected; ordinate: changes in perfusion pressure. Solid lines show the arteries from cholesterol-fed rabbits ($N=5$) and broken lines control animals ($N=4$). Vertical bars represent standard errors of mean values.

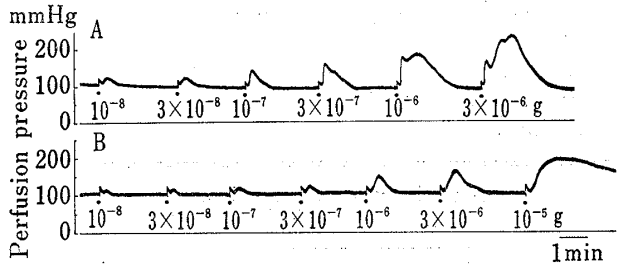

Fig. 3. Effects of Increasing Doses of Norepinephrine on Isolated Central Ear Arteries from Control (A) and Cholesterol-fed Rabbits (B)

rabbits and five for control animals were used. In general, the descending aortae were more sensitive to the agents than the ascending aortae. The application of norepinephrine, 3×10^{-9}—3×10^{-5} g/ml, caused greater contractions of the atherosclerotic aortae than those of the controls. The minimum effective doses, however, were similar in both groups. Angiotensin, 10^{-9}—3×10^{-6} g/ml, elicited greater contraction of atherosclerotic aortic strips than in the controls. A significant difference between the responses in the two groups was obtained in the doses shown in Fig. 4. A very poor responsiveness of the normal ascending aortae to angiotensin was observed. Histamine, 10^{-8}—3×10^{-4} g/ml, showed smaller contractions of the atherosclerotic aortae than those of the controls. The minimum effective dose in the atherosclerotic aortae was three times higher than that in the controls. On the other hand, no significant difference in the responses of both groups to potassium chloride, 7.5×10^{-4}—2.2×10^{-2} g/ml, was observed, although the contractions of the control aortae were slightly greater than those of the atherosclerotic aortae. Barium chloride, 3×10^{-5}—10^{-2} g/ml, showed a tendency to induce greater contractions of the atherosclerotic aortae than the controls but there was no significant difference in contractions of the two groups.

Discussion

The present study demonstrated that the smooth muscle of isolated vessels from cholesterol-fed rabbits showed different responses to the vasoactive agents depending on the blood vessels.

In Langendorff heart preparations the coronary arteries from cholesterol-fed rabbits showed the similar responses to glyceryl trinitrate and papaverine to those of control animals. This might be due to the fact that little atheroma was formed in the coronary arteries of

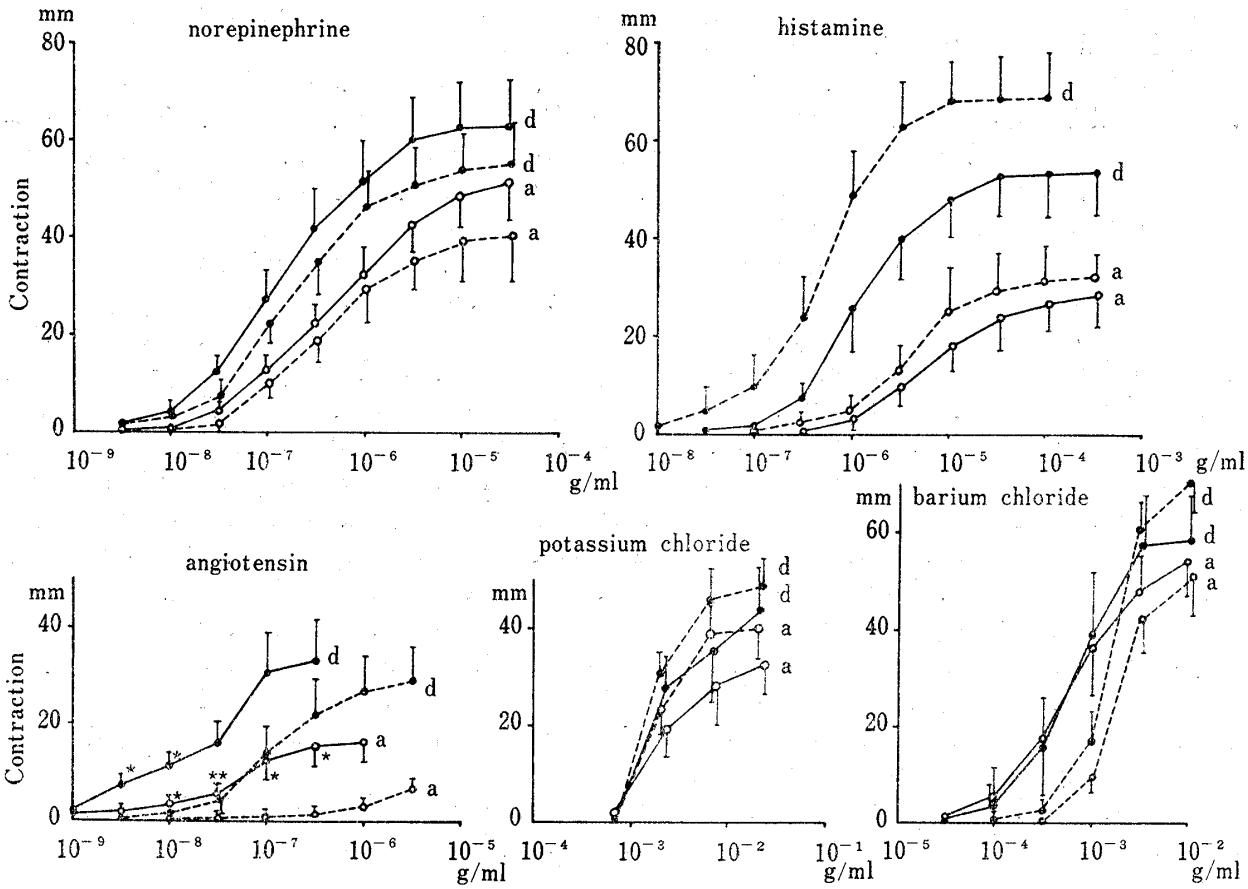

Fig. 4. Dose-response Curves for Norepinephrine, Histamine, Angiotensin, Potassium Chloride and Barium Chloride in the Strips of Ascending (a) and Descending Aortae (d) from Cholesterol-fed and Control Rabbits

Abscissa: doses of the agents in g/ml; ordinate: contraction of the strips in mm. Solid lines show cholesterol-fed rabbits ($N=7$) and broken lines control animals ($N=5$). Vertical bars represent standard errors of mean values.
*: statistically significant from corresponding control value ($p<0.05$) and
**: highly significant from corresponding control value ($p<0.01$).

cholesterol-fed rabbit hearts. In other words, too little damage may exist in them to alter the responses to the agents.

Various modifications of the smooth muscle cells and alterations of the elastic and ground substances in the atherosclerotic vessels have been reported.[8] The central ear arteries from cholesterol-fed rabbits, in which thick fatty tissue deposition in the perivascular tissue was observed, showed smaller contractile responses to norepinephrine, histamine and potassium chloride than those from control animals. The less responsiveness of the former arteries suggests an aggravation of contractile capacity of the smooth muscle because of the thickened connective tissue, reduction of pharmacological responsiveness in receptor sites to the agents, the amount of a given agent to reach to the receptors or metabolic derangement in the tissue. The changes in responsiveness of the vessels of the ear may be non-specific in nature, since the changes were observed in not only norepinephrine and histamine but also in potasium chloride.

Somlyo, *et al.*, using dog aortic strips, demonstrated a pharmacologically heterogenous smooth muscle cell population in the tissue.[9] There was a specific caudal increase in the sensitivity of aortic strips to vasopressin and norepinephrine between the aorta and bifuraction. It also is the case in rabbit aorta and to other agents, since in the present study the descending

8) A.P. Somlyo and A.V. Somlyo, *Pharmacol. Rev.*, **20**, 197 (1968).
9) A.V. Somlyo, R.L. Sandberg, and A.P. Somlyo, *J. Pharmacol. Exptl. Therap.*, **149**, 106 (1965).

aorta was consistently more sensitive to norepinephrine, histamine, angiotensin, potassium chloride and barium chloride than ascending aorta.

After the three-month feeding of cholesterol-containing pellet diets, atheromatous plaque was formed in the intima of thoracic aorta, especially in the ascending aorta. The atherosclerotic aortae showed greater responses to norepinephrine, angiotensin and barium chloride than the controls. On the contrary, histamine and potassium chloride induced smaller contractions of the atherosclerotic aorta than in the controls. Such various changes suggest that the atherosclerotic degeneration in the aortic intima influenced either favorably or unfavorably on the contraction of smooth muscle of the vessels depending on the agent.

It has been shown by many investigators in experimental animals and patients with hypertension that the responses to vasoactive agents vary depending upon the preparations, the level of the blood pressure and other various experimental conditions. The relationship between experimental hypertension and cholesterol feeding in rabbits has also been reported by several investigators. Campbell, *et al.* demonstrated that the development of atheroma in cholesterol-fed rabbits was not associated with any change in diastolic blood pressure.[10] A similar result was observed by Shapiro and Seecof.[11] On the other hand, Bronte-Stewart and Heptinstall[12] and Carrier, *et al.*[13] reported rises and falls in systolic blood pressure of cholesterol-fed rabbits, respectively. Although no measurement of blood pressure in the rabbits was done in the present study, no symptoms due to hypertension were noticed in cholesterol-fed rabbits. However, angiotensin, but not norepinephrine, elicited a significantly greater contraction of the atherosclerotic aortic strips than the controls. This suggest some relevance of atheroma formation to hypertension.

The detailed mechanism of the changes in contractile response of the blood vessels from cholesterol-fed rabbits to the vasoactive agents need further investigation.

10) D.J. Cambell, A.J. Day, S.L. Skinner, and R.K. Tume, *Atherosclerosis*, **18**, 301 (1973).
11) S. Shapiro and D.F. Seecof, *J. Lab. Clin. Med.*, **10**, 826 (1925).
12) B. Bronte-Stewart and R.H. Heptinstall, *J. Pathol. Bacteriol.*, **68**, 407 (1954).
13) O. Carrier, Jr., B.R. Clower, and P.J. Whitington, *J. Atheroscler. Res.*, **8**, 229 (1968).