Effect of Hypercholesterolemia on Vascular Reactivity in the Rabbit

I. Endothelium-Dependent and Endothelium-Independent Contractions and Relaxations in Isolated Arteries of Control and Hypercholesterolemic Rabbits

Tony J. Verbeuren, François H. Jordaens, Ludo L. Zonnekeyn, Cor E. Van Hove, Marie-Claire Coene, and Arnold G. Herman
From the Division of Pharmacology, Departments of Medicine and Pharmacy, Universitaire Instelling Antwerpen, University of Antwerp, Wilrijk, Belgium

SUMMARY. We studied the effects of hypercholesterolemia on vascular responsiveness in different arteries isolated from rabbits: control groups of rabbits and groups receiving the atherogenic diet consisted of eight animals each. In the arteries, 16 weeks of cholesterol-rich (0.3%) diet evoked intimal lesions which were more pronounced than those noted after 8 weeks of hypercholesterolemia; the aortic arch was affected significantly more by the lesions than the abdominal aorta and the pulmonary artery. Segments of the arteries were mounted in organ chambers for isometric tension recording or for measurement of the endothelium-derived relaxant factor. Contractions caused by acetylcholine and prostaglandin F\(_2\)\(_\alpha\) were not altered by the hypercholesterolemia; those evoked by serotonin were moderately augmented only in the aortic arch of hypercholesterolemic rabbits. As the degree of intimal lesion formation increased, the contractions to norepinephrine and clonidine were progressively inhibited. The endothelium-independent relaxations to nitroglycerin were inhibited in only the most severely affected arteries; the endothelium-dependent relaxations to acetylcholine and adenosine triphosphate were progressively inhibited as the degree of fatty streak formation augmented. Thus, in the aortic arch, the relaxations to \(3 \times 10^{-6} \) M acetylcholine, expressed as percent of the initial contraction, decreased from 86.7 ± 3.3% in control tissues to 16.3 ± 8.6% in the 16-week hypercholesterolemic vessels; in the abdominal aortas these relaxations averaged 93.5 ± 2.2% in control vessels and 72.0 ± 6.9% in the hypercholesterolemic tissues. The acetylcholine-induced release of endothelium-derived relaxant factor from the abdominal aorta was not significantly affected by the hypercholesterolemia. We conclude from these studies that in arteries obtained from hypercholesterolemic rabbits: (1) the contractions caused by serotonergic mechanisms tend to be augmented, while those to \(\alpha\)-adrenergic activation are decreased, (2) the endothelium-independent relaxations are modified only in the more severely affected arteries, and (3) the endothelium-dependent relaxations are progressively inhibited as the degree of fatty streak formation augments, probably because a step subsequent to the release of endothelium-derived relaxant factor is altered. (Circ Res 58: 552–564, 1986)

HYPERCHOLESTEROLEMIA is the most common risk factor associated with atherosclerosis in western society (see Faggiotto et al., 1984). Several studies have illustrated that atherosclerotic blood vessels are very susceptible to the development of vasospasm (Schroeder et al., 1977; Cipriano et al., 1979; Waters et al., 1983; see Heistad et al., 1984). Only a limited number of studies have been performed to investigate the vascular reactivity of hypercholesterolemic and atherosclerotic blood vessels (Yokoyama and Henry, 1979; Henry and Yokoyama, 1980; Rosendorff et al., 1981; Yokoyama et al., 1983; Heistad et al., 1984), and these investigations have focused on the contractile responsiveness of the blood vessels studied.

It recently became evident that several vasoactive substances (vasodilators, but also vasoconstrictor agents such as norepinephrine and serotonin) can cause relaxation of blood vessels by releasing a potent vasodilator substance, the endothelium-derived relaxant factor (EDRF) from the endothelial cells (Furchgott and Zawadzki, 1980; De Mey and Vanhoutte, 1981; Cohen et al., 1983; Griffith et al., 1984; Cocks and Angus, 1984). These observations have led to the proposal that dysfunction or denudation of the endothelium in atherosclerosis may cause impairment of the EDRF release, which then may lead to augmented vasoconstrictor responses (Vanhoutte, 1983; see also Heistad et al., 1984).

The purpose of the present investigation was to
compare relaxations and contractions of arteries obtained from hypercholesterolemic rabbits with those observed in arteries obtained from control rabbits, with the emphasis on the comparison of endothelium-dependent relaxations. Some of the results have been presented at the February 1985 meeting of the Belgian Physiological and Pharmacological Society in Brussels (Verbeuren et al., 1985) and at the Spring meeting of the British Pharmacological Society in Cardiff, U.K. (Coene et al., 1985).

Methods

In a first series of introductory experiments, two groups of eight male rabbits received either the atherogenic or the control diet for 8 weeks. For the major part of the study, the diets were administered to two groups of eight male New Zealand rabbits for 16 weeks. All animals received 150 g of the respective diet daily. Both the control and the atherogenic diets were obtained from Altromin (West Germany). The atherogenic diet was prepared by adding 0.45 g of cholesterol to 150 g of the commercially available diet; three separate determinations of the cholesterol content of the atherogenic diets showed that 0.28 ± 0.02% of cholesterol was present in the diet used for the 16-week experiments, and 0.27 ± 0.02% of cholesterol was present in the diet used for the 16-week experiments.

One group of male New Zealand rabbits (receiving control diet) was used to study the endothelium dependency of the vascular responses.

Tissue Preparation

After 8 or 16 weeks of receiving the respective diets, the rabbits were anesthetized with sodium pentobarbital (30 mg/kg, iv). A blood sample was obtained for determination of plasma cholesterol and triglyceride levels, and then the rabbits were killed. Segments of the aortic arch, the brachiocephalic artery, the thoracic aorta, the abdominal aorta, and the pulmonary artery were carefully removed from the aortic arch or to the abdominal aorta) were investigated histologically for the occurrence of fatty streaks. Cross-sections of the blood vessel segments, stained with the hematoxylin and eosin (HE) method or with the periodic acid-Schiff method (PAS reaction) were investigated. The part of the intimal outline covered with foam cell plaques was measured and expressed as a percentage of the total intimal outline of the section; this value was taken to represent the surface area covered with lesions. The thickness of the fatty streaks was then measured and expressed as a percentage of the unaffected wall thickness; since the thickness of the fatty streaks varied over the area affected, a mean value was estimated.

Evaluation of Fatty Streak Formation

Segments of the thoracic aorta (either adjacent to the aortic arch or to the abdominal aorta) were investigated histologically for the occurrence of fatty streaks. Cross-sections of the blood vessel segments, stained with the hematoxylin and eosin (HE) method or with the periodic acid-Schiff method (PAS reaction) were investigated. The part of the intimal outline covered with foam cell plaques was measured and expressed as a percentage of the total intimal outline of the section; this value was taken to represent the surface area covered with lesions. The thickness of the fatty streaks was then measured and expressed as a percentage of the unaffected wall thickness; since the thickness of the fatty streaks varied over the area affected, a mean value was estimated.

Evaluation of the Presence of the Endothelium

Segments of the four arteries obtained from control and 16-week cholesterol rabbits, were opened longitudinally and stained in vitro with AgNO₃ (Caplan et al., 1974; see De Mey and Vanhoutte, 1981). Briefly, the opened segments were mounted on pieces of cork and stained in the dark with AgNO₃ (24 mm) in the presence of glucose (233 mm) and Hepes buffer (20 mm) at pH 7.4 during 60 seconds. After being rinsed with glucose solution, the tissues were fixed (1 hr) with glutaraldehyde (3%) in 0.1 M sodium cacodylate buffer. The fixed tissues were dehydrated, embedded in DPX (Fluka), and their luminal surface was examined by light microscopy.

Organ Chamber Experiments

Segments (3 mm long) of the arteries used were mounted in organ chambers (50 ml) filled with physiological salt solution (37°C), as described previously (De Mey and Vanhoutte, 1980; Verbeuren et al., 1983, 1985). The isometric tension development was monitored continuously by means of force transducers (Statham UC2). After equilibration (30 min), the arterial segments were placed at the optimal point of their length-tension relationship, using a standard concentration of norepinephrine (3 × 10⁻⁶ m; see Vanhoutte and Leusen 1969). The preparations were then allowed to equilibrate at their optimal length for 45 minutes before experimentation. In some artery segments obtained from control rabbits, the endothelium was mechanically removed by gently rubbing the intimal surface of the vessel with a cotton swab (Furchgott and Zawadzki, 1980; De Mey and Vanhoutte, 1981). After equilibration, two major experimental protocols were used in the present study.

Protocol Used in the Introductory Studies

These studies were performed on arteries obtained from control rabbits and from rabbits fed the cholesterol-rich diet for 8 weeks. In one segment of each of the three arteries (aortic arch, abdominal aorta, and pulmonary artery) prepared from one rabbit, the following experimental protocol was performed: (1) a dose-response curve to acetylcholine (10⁻⁶ to 3 × 10⁻⁵ m) during a contraction caused by norepinephrine (3 × 10⁻⁷ m); (2) a dose-response curve...
curve to adenosine triphosphate (ATP) (10^-8 to 10^-4 M) during a contraction caused by norepinephrine (3 x 10^-7 M); (2) a dose-response curve to serotonin (10^-8 to 10^-5 M); and (3) a dose-response curve to prostaglandin F_2alpha (2 x 10^-6 M).

Protocol Used for the Major Part of the Study

These studies were performed on arteries obtained from control rabbits (segments with or without endothelium) and rabbits fed cholesterol for 16 weeks. Two segments of each of the four arteries used (aortic arch, abdominal aorta, pulmonary artery, and brachiocephalic artery) were prepared from each animal. Before the start of the experimental protocol, indomethacin (3 x 10^-5 M) was administered to the preparations and remained present throughout the duration of the experiments, since it has been shown recently that endogenous prostaglandins can modulate the vascular reactivity of rabbit blood vessels (Förstermann et al., 1984). The following experimental protocol was performed.

In Segment a: (1) a dose-response curve to acetylcholine (10^-10 to 10^-4 M) during a contraction caused by prostaglandin F_2alpha (2 x 10^-4 M); (2) a dose-response curve to acetylcholine (10^-9 to 10^-4 M); (3) a dose-response curve to norepinephrine (10^-10 to 10^-5 M); and (4) a dose-response curve to clonidine (10^-8 to 10^-3 M).

In Segment b: (1) a dose-response curve to ATP (10^-8 to 10^-4 M) during a contraction caused by prostaglandin F_2alpha (2 x 10^-4 M); (2) a dose-response curve to nitroglycerin (10^-5 to 10^-3 M) during a contraction caused by prostaglandin F_2alpha (2 x 10^-4 M); (3) a dose-response curve to serotonin (10^-9 to 10^-5 M); (4) a contraction to norepinephrine (10^-5 M); and (5) a dose-response curve to prostaglandin F_2alpha (3 x 10^-7 to 10^-5 M).

In a separate set of experiments, in the four arteries obtained from control rabbits we tested the endothelium dependency of the different responses described above by comparing a control and a rubbed segment of each artery. In some of these experiments, the protocols as described for segments a and b were used. A major difference between the 8-week and the 16-week hypercholesterolemic tissues and their respective control tissues was that, in the 8-week group, the relaxations to acetylcholine and ATP were tested during contractions caused by norepinephrine (3 x 10^-7 M), whereas in the 16-week group, prostaglandin F_2alpha (2 x 10^-6 M) was used to obtain a contractile response. Neither the amplitude of the contractions nor the relaxations obtained in the respective control tissues were significantly different for the aortic arch and the abdominal aorta. For the pulmonary artery, despite the fact that the contractile response to norepinephrine was significantly larger than that to prostaglandin F_2alpha, the relaxations to acetylcholine and ATP were similar in both control groups (see Table 9).

Bioassay of Endothelium-derived Relaxant Factor

In segments of abdominal aortas obtained from control and 16-week hypercholesterolemic rabbits, the release of EDRF caused by acetylcholine was measured by a bioassay technique comparable to that described by Rubanyi et al. (1985); see also Griffith et al. (1984).

Briefly, two arterial segments (5 cm long), one obtained from a control, the other obtained from a hypercholesterolemic rabbit, were mounted vertically in an organ chamber filled with aerated physiological salt solution containing phenolamine (3 x 10^-6 M), propranolol (10^-6 M), and indomethacin (3 x 10^-5 M) at 37°C; parallel to the arteries, a piece of tubing was mounted (see Fig. 1). The arteries and the piece of tubing were perfused continuously with physiological salt solution containing the same cocktail at 3 ml/min entering at the upper part of the segments. To start the experiments, the solution perfusing through the tubing dripped directly onto a segment of an aortic arch (obtained from the control rabbit); in this segment, the endothelium had been mechanically removed as described above. This "detector" tissue was mounted vertically over two stainless steel hooks, and its isometric tension development was monitored continuously (see Fig. 1). The initial basal tension of the detector artery was set at 10 g. Into the three circuits, drugs could be infused by means of infusion pumps. Drugs could also be added directly to the perfusing physiological salt solution. By moving the organ chamber, the outlet of the arterial segments could be placed above the detector tissue. The distance between the outlet of the arteries and the detector tissue was kept as short as possible (2 cm), since it has been established that the EDRF has a half-life of only about 6 seconds.

![Schematic representation of the experimental set-up used to measure the release of the endothelium-derived relaxant factor (EDRF) from abdominal aorta segments obtained from control and hypercholesterolemic rabbits. The bioassay artery was a segment of the aortic arch obtained from a control rabbit; in this segment, the endothelium had been mechanically removed. The Krebs-Ringer solution used to perfuse the aortas and the organ bath solution contained indomethacin (3 x 10^-5 M), phenolamine (3 x 10^-6 M) and propranolol (10^-6 M), in order to rule out the involvement of prostaglandins or of adrenergic mechanisms.](image-url)
(Griffith et al., 1984; Rubanyi et al., 1985). Before the start of the actual experiments, the tissues were allowed to equilibrate for 45 minutes in the perfusion chambers.

In introductory experiments \( (n = 5) \), two abdominal aorta segments (5 cm long), one with and one without endothelium, obtained from control rabbits, were mounted in the bioassay set-up; a rubbed segment of an aortic arch was used as bioassay tissue. When prostaglandin \( F_2 \) \( (2 \times 10^{-6} \text{ M}) \) was added to the perfusing physiological salt solution, it caused a sustained contraction of the bioassay tissue \( (5.2 \pm 1.7 \text{ g}) \). During this contraction, the efflux of the aortas with or without endothelium was allowed to drip over the bioassay tissue; this did not result in a significant change in the contractile response to prostaglandin \( F_2 \) \( (96.6 \pm 3.5\% \text{ of the control}) \). Perfusion of acetylcholine \( (1.3 \times 10^{-5} \text{ M}) \) directly over the bioassay tissue did not alter the contractile response of this tissue to prostaglandin \( F_2 \) \( (96.6 \pm 3.5\% \text{ of the control response}) \). If the efflux of the aorta without endothelium perfused with acetylcholine was assayed, then the contractile response of the bioassay tissue was not significantly altered \( (100.4 \pm 6.7\% \text{ of control}) \). However, if the efflux of the aorta with endothelium perfused with acetylcholine was assayed, then the contractile response of the bioassay tissue significantly decreased to 73.8 \( \pm 8.1\% \text{ of the control}) \). These experiments illustrate that, during low-flow perfusion of the rabbit abdominal aorta with endothelium, acetylcholine releases a relaxing factor into the perfusate, and thus confirm earlier bioassay experiments performed on rabbit and dog arteries (Furchgott and Zawadzki, 1980; Griffith et al., 1984) and the canine femoral artery (Rubanyi et al., 1985).

**Drugs**

The following pharmacological agents were used: acetylcholine (Sigma), ATP (Sigma), clonidine (Boehringer-Ingelheim), indomethacin (Merck, Sharp & Dohme), norepinephrine (Sigma), phenolamine (Ciba-Geigy), propranolol (ICI), prostaglandin \( F_2 \) (Upjohn), and serotonin (Janssen Chimica).

**Statistical Analysis**

The data are expressed as means \( \pm \text{SEM} \). For statistical analysis, Student’s \( t \)-test for paired or unpaired observations was used: \( P \) values smaller than 0.05 were considered to be significant. For the contractile agents, the potency of the compounds was evaluated by calculating \( ED_{50} \) values. The \( ED_{50} \) values were determined graphically after linear regression of the 20–80% region of the dose-response curves (Tallarida and Murray, 1981).

**Table 1**

**Body Weight, Plasma Cholesterol, and Plasma Triglyceride Levels in Control and Hypercholesterolemic Rabbits**

<table>
<thead>
<tr>
<th>Artery</th>
<th>Control (8 wk)</th>
<th>Cholesterol-rich (8 wk)</th>
<th>Control (16 wk)</th>
<th>Cholesterol-rich (16 wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cholesterol (mg/dl)</td>
<td>34 ( \pm ) 4</td>
<td>958 ( \pm ) 125\†</td>
<td>31 ( \pm ) 5</td>
<td>1080 ( \pm ) 112\†</td>
</tr>
<tr>
<td>Plasma triglycerides (mg/dl)</td>
<td>72 ( \pm ) 10</td>
<td>115 ( \pm ) 33</td>
<td>160 ( \pm ) 46</td>
<td>229 ( \pm ) 39</td>
</tr>
<tr>
<td>Body wt (g)</td>
<td>2366 ( \pm ) 68</td>
<td>2364 ( \pm ) 76</td>
<td>2550 ( \pm ) 67</td>
<td>2477 ( \pm ) 66</td>
</tr>
<tr>
<td>Increase in body wt (g)</td>
<td>1281 ( \pm ) 74</td>
<td>1296 ( \pm ) 98</td>
<td>1793 ( \pm ) 80</td>
<td>2167 ( \pm ) 77\†</td>
</tr>
</tbody>
</table>

\* Values shown as means \( \pm \text{SEM} \).

\† Value significantly different from respective control \( (P < 0.05; \text{Student’s} \ t\text{-test for unpaired observations}) \). ND = not determined.

**Table 2**

**Macroscopic Evaluation of Fatty Streak Formation in Arteries of Control and Hypercholesterolemic Rabbits**

<table>
<thead>
<tr>
<th>Artery</th>
<th>Control</th>
<th>8-Wk cholesterol</th>
<th>16-Wk cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic arch</td>
<td>0</td>
<td>4.0 ( \pm ) 0.5</td>
<td></td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>0</td>
<td>2.4 ( \pm ) 0.4</td>
<td></td>
</tr>
<tr>
<td>Brachiocephalic artery</td>
<td>0</td>
<td>3.6 ( \pm ) 0.5</td>
<td></td>
</tr>
<tr>
<td>Pulmonary artery</td>
<td>0</td>
<td>2.4 ( \pm ) 0.3</td>
<td></td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td>2.2 ( \pm ) 0.6</td>
<td>2.8 ( \pm ) 0.6</td>
<td></td>
</tr>
</tbody>
</table>

* Scale: 0–5

0: no visible fatty streaks
1: only a few, small, widely spread fatty streaks
2: a few fatty streaks spread over the intimal surface
3: a lot of small fatty streaks which do not overlap
4: fatty streaks separated by little spots of "intact" intimal surface
5: the total intimal surface of the artery is covered by fatty streaks

† For the hypercholesterolemic tissues, means \( \pm \text{SEM} \) of at least seven arteries are shown.

‡ Value significantly lower than that obtained for the aortic arch \( (P < 0.05; \text{Student’s} \ t\text{-test for unpaired observations}) \). ND = not determined.

**Results**

**Body Weight, Plasma Cholesterol, and Triglyceride Levels**

The body weight of the rabbits at the start of the experiments was the same for the four groups used; the weight of the 16-week cholesterol group was increased significantly more than that of the control group (Table 1). Both in the 8-week- and in the 16-week-cholesterol group, the total plasma cholesterol levels were markedly increased as compared to the control levels; the plasma triglyceride concentrations were not significantly affected by the hypercholesterolemia (Table 1).

**Macroscopic and Microscopic Examination of the Intimal Surface of the Isolated Blood Vessels**

Table 2 illustrates that no visible lesions were noted in any of the control blood vessels studied. Of the arteries obtained from the rabbits fed the cholesterol-rich diet for 16 weeks, the aortic arches were significantly more affected by the lesions than the abdominal aortas and the pulmonary arteries.
Table 3

Microscopic Evaluation of Fatty Streak Formation in Thoracic Aortas of Hypercholesterolemic Rabbits

<table>
<thead>
<tr>
<th></th>
<th>8-Wk cholesterol</th>
<th>16-Wk cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>((n = 4))</td>
<td>((n = 5))</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjacent to aortic arch</td>
<td>65 ± 12</td>
<td>34 ± 15</td>
</tr>
<tr>
<td>Adjacent to abdominal aorta</td>
<td>33 ± 18</td>
<td>28 ± 12</td>
</tr>
<tr>
<td>Surface area†</td>
<td>80 ± 11</td>
<td>83 ± 9§</td>
</tr>
<tr>
<td>Thickness‡</td>
<td>66 ± 17</td>
<td>80 ± 15§</td>
</tr>
</tbody>
</table>

* Values shown as means ± SEM; none of the control tissues examined showed any significant fatty streak formation.
† Surface area damaged is shown as percent of total surface area.
‡ Thickness of the fatty streak is expressed as percent of the total wall thickness.
§ Thickness is significantly increased as compared to that noted after 8 weeks of hypercholesterolemia \((P < 0.05; \text{Student's } t\text{-test for unpaired observations})\).

Microscopic evaluation of the fatty streak formation performed on segments of thoracic aortas (one segment adjacent to the aortic arch and one segment adjacent to the abdominal aorta) is shown in Table 3. In none of the control segments was any degree of lesions measured. Although the surface area affected by the intimal lesions was not significantly increased when aortas from 16-week hypercholesterolemic rabbits were compared with those obtained after 8 weeks of cholesterol feeding (see Tables 2 and 3), the thickness of the fatty streaks significantly augmented with the time the rabbits were fed the cholesterol-rich diet. The degree of lesions as determined with the microscopic examinations of the fatty streak formation correlated significantly with the lesions observed in thoracic aorta-segments as evaluated by the macroscopic examination.

After staining the intimal surface of the arterial segments with AgNO₃, the typical mosaic pattern of silver lines, considered to represent the borders of adjacent endothelial cells, was detected in all the arteries investigated. A typical example is shown in Figure 2 for the abdominal aorta of a control (panel A) and a hypercholesterolemic (panel B) rabbit. The only difference detected was that the endothelial cells of the hypercholesterolemic arteries seem to be changed in shape, compared to those of control arteries (see Fig. 2).

Endothelium Dependency of Vascular Responses in the Isolated Arteries

Increasing concentrations of acetylcholine \((10^{-9} \text{ to } 10^{-4} \text{ M})\), prostaglandin \(F_2\alpha\) \((10^{-7} \text{ to } 3 \times 10^{-5} \text{ M})\), norepinephrine \((10^{-8} \text{ to } 10^{-3} \text{ M})\), clonidine \((10^{-8} \text{ to } 10^{-5} \text{ M})\), and serotonin \((10^{-8} \text{ to } 10^{-5} \text{ M})\) evoked concentration-dependent contractions in control segments of the aortic arch and the abdominal aorta; the concentration-dependent relaxations to acetylcholine were abolished, those to ATP were markedly attenuated, and those to nitroglycerin were unaffected in the arteries denuded of the endothelium, as illustrated in Figure 4 (aortic arch) and Figure 5 (abdominal aorta). Similar results were obtained in segments of the brachiocephalic and pulmonary arteries (data not shown).

Vascular Responses in Hypercholesterolemic Arteries

Rabbits fed the Cholesterol-rich Diet for 8 Weeks

The contractions caused by serotonin \((10^{-8} \text{ to } 10^{-5} \text{ M})\) in segments of the aortic arch and the abdominal aorta obtained from rabbits fed cholesterol for 8 weeks are not different from those obtained in arteries of control rabbits, as illustrated by the ED₅₀ values and maximal contractions (Tables 4 and 5).

In the aortas obtained from the 8-week hypercholesterolemic rabbits, acetylcholine \((10^{-9} \text{ to } 3 \times 10^{-6} \text{ M})\) and ATP \((10^{-8} \text{ to } 10^{-4} \text{ M})\) evoked concentration-dependent relaxations; these relaxations were significantly attenuated, compared to those noted in the control arteries (Fig. 4 and 5). In pulmonary arteries, the relaxations to acetylcholine and ATP were not affected by the cholesterol-rich diet (data not shown).

Rabbits Fed the Cholesterol-rich Diet for 16 Weeks

Constrictions. Increasing concentrations of prostaglandin \(F_2\alpha\) \((10^{-7} \text{ to } 3 \times 10^{-5} \text{ M})\), acetylcholine \((10^{-9} \text{ to } 10^{-4} \text{ M})\), norepinephrine \((10^{-8} \text{ to } 10^{-5} \text{ M})\), clonidine \((10^{-8} \text{ to } 10^{-5} \text{ M})\), and serotonin \((10^{-8} \text{ to } 10^{-5} \text{ M})\) evoked constrictions in segments of the aortic arch (Fig. 3; Table 4) and the abdominal aorta (Table 5) obtained from rabbits fed the cholesterol-rich diet for 16 weeks. The contractile responses to prostaglandin \(F_2\alpha\) and acetylcholine were not different from those obtained in control tissues with endothelium (Tables 4 and 5). For serotonin, the only sig-
significant difference obtained was an increased contraction to the lowest concentrations of the indoleamine in the aortic arch (Fig. 3); this difference was not reflected in a significantly decreased ED$_{50}$ value (Table 4). In the aortic arch, but not in the abdominal aorta, a significantly augmented ED$_{50}$ value for norepinephrine and clonidine were obtained after 16 weeks of hypercholesterolemia; in the aortic arch, the contractile responses to lower and moderate concentrations of the $\alpha$-adrenergic agonists were significantly decreased (Fig. 3; Tables 4 and 5). The results obtained with the brachiocephalic and pulmonary arteries confirm these observations (data not shown).

Relaxations. In the segments of the aortic arch obtained from 16-week hypercholesterolemic rabbits, acetylcholine (10$^{-9}$ to 3 x 10$^{-6}$ M) no longer evoked significant relaxations (Fig. 4). The relaxations to ATP (10$^{-8}$ to 10$^{-4}$ M) and nitroglycerin (10$^{-4}$ to 10$^{-5}$ M) were markedly attenuated, compared to the relaxations noted in the control tissues; however complete relaxation could still be obtained with the highest concentration of nitroglycerin (Fig. 4). In segments of the abdominal aorta isolated from the 16-week hypercholesterolemic rabbits, the relaxations to acetylcholine and ATP were significantly attenuated, compared to those observed in the control tissues; the relaxations to nitroglycerin however, were not different from those noted in the control blood vessels (Fig. 5). The shifts of the dose-response curves to acetylcholine and ATP noted in the 16-week hypercholesterolemic aortas were more pronounced than those obtained in the 8-week hypercholesterolemic arteries (Figs. 4 and 5). The data obtained in the brachiocephalic and pulmonary arteries are in agreement with those described for the aortas (data not shown). The magnitude of the maximal relaxations obtained to acetylcholine in all four arteries studied correlated significantly with the degree of visual lesions observed in the preparations; thus, less relaxation was obtained in the more severely affected arteries (Fig. 6).

Measurement of Endothelium-Derived Relaxant Factor Release

In four segments of abdominal aortas obtained from either 16-week control or hypercholesterolemic rabbits, the release of EDRF evoked by two concen-
trations of acetylcholine (5 × 10⁻⁷ and 1.3 × 10⁻⁶ M) was investigated. In the bioassay tissue, continuous addition of prostaglandin F₂α (2 × 10⁻⁶ M) to the perfusing physiological salt solution caused sustained contractions which averaged 4.5 ± 0.5 g. If, during these contractions, the efflux of unstimulated control or hypercholesterolemic arteries was allowed to drip over the bioassay tissue, then a non-significant slight decrease of the contraction to 96.9 ± 3.1% and 94.3 ± 4.1%, respectively, was obtained.

Perfusion of acetylcholine (5 × 10⁻⁷ M or 1.3 × 10⁻⁶ M) directly onto the bioassay tissue did not significantly alter the contractile response of this tissue to prostaglandin F₂α (100.8 ± 0.5% and 107.5 ± 3.7% of the control response, respectively). If the efflux of the control and hypercholesterolemic aortas perfused with acetylcholine was assayed, then significant concentration-dependent relaxations were obtained in the bioassay tissue (Fig. 7). No significant differences were noted when the relaxations caused by the efflux of control and hypercholesterolemic aortas were compared (Fig. 7). At the end of each experiment, the effect of nitroglycerin (10⁻⁷ M) on the bioassay aortic arch was examined during contractions caused by prostaglandin F₂α (2 × 10⁻⁶ M); the compound significantly decreased these contractions (4.7 ± 0.7 g) to 28.8 ± 4.7% of the control response.

### Table 4

<table>
<thead>
<tr>
<th></th>
<th>Control rabbits With endothelium</th>
<th>Control rabbits Without endothelium</th>
<th>Rabbits fed cholesterol-rich diet</th>
<th>8 wk</th>
<th>16 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum response (g)</td>
<td>6.0 ± 0.8 (20)</td>
<td>7.3 ± 2.1 (7)</td>
<td>5.2 ± 0.8 (7)</td>
<td>5.4 ± 0.7 (8)</td>
<td></td>
</tr>
<tr>
<td>Serotonin</td>
<td>10.2 ± 1.2 (20)</td>
<td>9.0 ± 1.1 (7)</td>
<td>7.5 ± 1.3 (8)</td>
<td>6.2 ± 0.4 (8)</td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>5.9 ± 0.8 (20)</td>
<td>5.8 ± 1.3 (7)</td>
<td>6.3 ± 0.5 (3)</td>
<td>5.6 ± 1.2 (7)</td>
<td></td>
</tr>
<tr>
<td>Clonidine</td>
<td>7.1 ± 1.4 (18)</td>
<td>6.3 ± 1.1 (7)</td>
<td>0.57 ± 0.2 (19)</td>
<td>0.96 ± 0.27 (8)</td>
<td></td>
</tr>
<tr>
<td>Prostaglandin F₂α</td>
<td>2.8 ± 0.2 (20)</td>
<td>0.55 ± 0.19 (7)</td>
<td>2.7 ± 0.5 (2)</td>
<td>2.0 ± 0.4 (8)</td>
<td></td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>4.15 ± 0.85</td>
<td>2.81 ± 0.90</td>
<td>5.6 ± 2.12</td>
<td>2.25 ± 0.34</td>
<td></td>
</tr>
<tr>
<td>ED₅₀ value (X 10⁻⁷ M)</td>
<td>1.10 ± 0.26</td>
<td>2.27 ± 0.86</td>
<td>2.01 ± 0.35†</td>
<td>23.5 ± 4.9†</td>
<td></td>
</tr>
<tr>
<td>Serotonin</td>
<td>6.60 ± 1.75</td>
<td>6.80 ± 1.30</td>
<td>33.6 ± 6.5</td>
<td>243 ± 47</td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>28.3 ± 10.2</td>
<td>29.5 ± 11.4</td>
<td>292 ± 85</td>
<td>367 ± 57</td>
<td></td>
</tr>
<tr>
<td>Clonidine</td>
<td>23.5 ± 4.9†</td>
<td>23.5 ± 4.9†</td>
<td>23.5 ± 4.9†</td>
<td>23.5 ± 4.9†</td>
<td></td>
</tr>
<tr>
<td>Prostaglandin F₂α</td>
<td>0.96 ± 0.27 (8)</td>
<td>2.25 ± 0.34</td>
<td>2.25 ± 0.34</td>
<td>2.25 ± 0.34</td>
<td></td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>2.01 ± 0.35†</td>
<td>23.5 ± 4.9†</td>
<td>23.5 ± 4.9†</td>
<td>23.5 ± 4.9†</td>
<td></td>
</tr>
</tbody>
</table>

* Values shown as means ± SEM; the number of experiments in each group is shown in parentheses.
† Value significantly different from that noted in the control tissues (with endothelium); P < 0.05; Student's t-test for unpaired observations.
TABLE 5

Evaluation of Contractile Responses in the Abdominal Aorta of Control and Hypercholesterolemic Rabbits*

<table>
<thead>
<tr>
<th></th>
<th>Control rabbits</th>
<th>Rabbits fed cholesterol-rich diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With endothelium</td>
<td>Without endothelium</td>
</tr>
<tr>
<td>Maximum response (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serotonin</td>
<td>5.7 ± 1.4 (20)</td>
<td>7.6 ± 2.4 (7)</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>8.5 ± 1.4 (20)</td>
<td>7.9 ± 2.6 (7)</td>
</tr>
<tr>
<td>Clonidine</td>
<td>4.9 ± 1.4 (20)</td>
<td>5.8 ± 1.5 (7)</td>
</tr>
<tr>
<td>Prostaglandin F^</td>
<td>6.3 ± 2.0 (18)</td>
<td>6.5 ± 2.5 (7)</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>0.79 ± 0.52 (20)</td>
<td>1.68 ± 0.38 (7)</td>
</tr>
<tr>
<td>EDM value (× 10^-7 m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serotonin</td>
<td>4.85 ± 0.95</td>
<td>3.50 ± 0.66</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>1.17 ± 0.35</td>
<td>0.79 ± 0.22</td>
</tr>
<tr>
<td>Clonidine</td>
<td>11.2 ± 3.2</td>
<td>7.47 ± 2.50</td>
</tr>
<tr>
<td>Prostaglandin F^</td>
<td>40.0 ± 10.0</td>
<td>27.5 ± 3.3</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>255 ± 90</td>
<td>207 ± 62</td>
</tr>
</tbody>
</table>

* Values shown as mean ± SEM; the number of experiments in each group is shown in parentheses.

Discussion

The major goal of the present study was to evaluate the effects of a cholesterol-rich diet on the vascular responsiveness of isolated rabbit arteries, with the emphasis on the influence of hypercholesterolemia on endothelium-dependent relaxations. Since the original observation by Furchgott and Zawadzki (1980), that several vasodilator substances cause relaxation of isolated blood vessels by evoking release of the "endothelium-derived relaxant factor" from the endothelial cells, it has been speculated that this mechanism might be impaired during diseases such as hypertension or atherosclerosis (see Vanhoutte, 1983; Heistad et al., 1984). Until the start of our study, only a limited amount of data regarding contractile responses in isolated blood vessels obtained from atherosclerotic animals was available (Henry and Yokoyama, 1980; Yokoyama et al., 1983, see Heistad et al., 1984). Therefore, in our study, both contractile responses and relaxations were compared in arteries obtained from either control and hypercholesterolemic arteries.

Since preliminary data from our laboratory indicated that significant intimal lesions of the arteries could already be detected after feeding rabbits a diet containing 0.3% of cholesterol (Beetens et al., 1985), we selected this "rather low" amount of daily cholesterol intake, and fed the rabbits a cholesterol-rich diet for either 8 or 16 weeks. Table 1 indicates that,
in the rabbits fed the cholesterol-rich diet, a marked increase in the total plasma cholesterol-levels was obtained. Even after 16 weeks, this increase was still lower than that obtained by feeding rabbits 2% cholesterol for 9–10 weeks (Henry and Yokoyama, 1980). An interesting observation is the significantly greater gain in body weight observed in the rabbits fed the cholesterol-rich diet for 16 weeks, which may be caused by a larger incorporation of lipid materials into various body tissues.

Although it was not a main objective of the pres-

FIGURE 5. Relaxations obtained with increasing concentrations of acetylcholine, nitroglycerin and ATP in abdominal aortas of control or hypercholesterolemic rabbits. O, control aorta with endothelium; ▽, control aorta without endothelium; □, 8 weeks hypercholesterolemic aorta; △, 16 weeks hypercholesterolemic aorta. Filled symbols (◇, ◆, ▲) indicate that the inhibition of the relaxations is significant (P < 0.05; Student's t-test for unpaired observations). The results are expressed as percent of the initial contractile response and are shown as means (n = at least 6 in each group).

FIGURE 6. Correlation between the maximum relaxation evoked by acetylcholine and the degree of fatty streak formation (as determined by visual inspection) in control arteries and arteries obtained from 16-week hypercholesterolemic rabbits. For the scale of the visual damage, see legend of Table 2. The response to acetylcholine is expressed as percent of the initial contraction caused by prostaglandin F2α (2 × 10⁻⁶ M). The correlation obtained was significant. Note that 17 of the unlesioned control arteries relaxed completely to acetylcholine.

FIGURE 7. Release of EDRF in abdominal aortas of control and 16-week hypercholesterolemic rabbits. The release of EDRF is evaluated by determining the relaxation of the aortic arch without endothelium (bioassay tissue) which was contracted with prostaglandin F2α (2 × 10⁻⁶ M) in presence of indomethacin (3 × 10⁻⁶ M), phentolamine (3 × 10⁻⁶ M), and propranolol (10⁻⁶ M). Shown is the response of the bioassay tissue (as percent of the initial contractile response to prostaglandin F2α) to increasing concentrations of EDRF, evoked by acetylcholine (5 × 10⁻⁷ M and 1.3 × 10⁻⁶ M) in the abdominal aortas. The results are shown as means ± SEM (n = 4). Acetylcholine evoked similar release of EDRF in abdominal aortas obtained from control and hypercholesterolemic rabbits.
ent study to investigate in detail the morphological changes caused by the cholesterol-rich diet, we have, besides visual investigation of the different arteries used, checked histologically the degree of fatty streak formation induced in segments of the thoracic aorta. Visual inspection of the arterial segments (Table 2) indicates that in the 16-week hypercholesterolemic rabbits, the formation of lesions was more pronounced in the aortic arch than in the abdominal aorta and the pulmonary artery. The microscopic evaluation performed on the thoracic aorta indicates that the thickness of the intimal lesions is significantly more pronounced after 16 weeks than after 8 weeks of hypercholesterolemia. Our results thus show that the cholesterol-rich diet fed to the rabbits caused intimal lesions in the arteries investigated, and they also confirm that the severity of these lesions can vary depending on the anatomical localization of the blood vessel studied (Kottke and Subbiah, 1978; see also, Faggiotto et al., 1984).

In segments of arteries obtained from control rabbits, the contractile responses to serotonin, norepinephrine, clonidine, prostaglandin F2, and acetylcholine were not altered if the endothelium was mechanically removed by gentle rubbing of the intimal surface. Although both decreased contractions to norepinephrine (De Mey and Vanhoutte, 1982) and increased contractile responses to norepinephrine, serotonin, and clonidine (Cohen et al., 1983; Cocks and Angus, 1984; Eglême et al., 1984) have been reported for denuded arteries of dogs and rats, our results are in agreement with those described by Furchgott (1983) illustrating an unaltered sensitivity to norepinephrine in rubbed segments of the rabbit aorta.

Hypercholesterolemia did not alter the contractile responses of the arterial segments to acetylcholine and prostaglandin F2, the contractions caused by acetylcholine are caused by activation of muscarinic receptors located on the smooth muscle cells and are inhibited by Ca++ entry blockers (see Vanhoutte, 1985), whereas those to prostaglandin F2 are, at least in part, independent of extracellular Ca++. (Rooke et al., 1984). Thus, the cholesterol-rich diet did not influence the responsiveness of the vascular smooth muscle cells to stimuli acting via different mechanisms, suggesting that—under the experimental conditions imposed—their contractile machinery was not altered. A similar conclusion was reached by Henry and Yokoyama (1980) who demonstrated that the contractile responses to KCl were not significantly altered in aortas of hypercholesterolemic rabbits.

Henry and Yokoyama (1980) and Yokoyama et al. (1983) have reported a decreased responsiveness to serotonin in aortas from rabbits fed a 2% cholesterol-rich diet for 10 weeks, and in aortas from rabbits with hereditary hyperlipidemia. In coronary arteries of the latter, in which no macroscopically detectable lesions could be observed, no increased contractile responses to serotonin were detected (Yokoyama et al., 1983). The results obtained with serotonin in our present investigation are in agreement with these earlier-obtained findings; indeed, after 16 weeks of hypercholesterolemia, the contractile responses of the most severely affected tissue, the aortic arch, showed an augmented responsiveness to lower concentrations of the indoleamine. Taken together with the results obtained with acetylcholine and prostaglandin F2, our findings seem to indicate that in arteries with severe atherosclerotic lesions, only the contractions caused by stimulation of the vascular serotonin-receptors are augmented; an enhanced number of serotonin receptors, as has been suggested in a preliminary report by Nanda and Henry (1982) for atherosclerotic aortas, may help explain these results.

In contrast to the findings with serotonin, the contractile responses to the alpha-2-adrenergic agonist norepinephrine are decreased in the arteries obtained from the 16-week hypercholesterolemic rabbits; this reduction is reflected by significantly increased ED50 values in pulmonary and brachiocephalic arteries and in aortic arches. These results are different from findings previously reported in "in vivo" models of hypercholesterolemia: indeed, in coronary blood vessels of anesthetized hypercholesterolemic dogs and in small resistance vessels of anesthetized hypercholesterolemic monkeys, augmented vasoconstrictor responses to norepinephrine have been reported (Rosendorff et al., 1981; Heistad et al., 1984). Moreover, in aortas of rabbits fed a cholesterol-rich diet, an increased number of alpha-adrenergic receptors has been detected (Nanda and Henry, 1982). It should be noted, however, that despite this increased number of alpha-adrenergic receptors, the contractions to phenylephrine noted in hypercholesterolemic rabbit aortas were not significantly augmented (Henry and Yokoyama, 1980; Yokoyama et al., 1983). Besides a decreased responsiveness to norepinephrine, the hypercholesterolemic arteries also showed significantly less responsiveness to clonidine. The finding that the contractions caused by the more specific alpha-adrenergic agonist phenylephrine are not altered (Henry and Yokoyama, 1980; Yokoyama et al., 1983), whereas those to the more specific alpha-adrenergic agonist clonidine and those to the nonspecific alpha-adrenergic agonist norepinephrine are decreased (as shown in the present study) in arteries obtained from hypercholesterolemic rabbits, may indicate that, in the hypercholesterolemic arteries, there is decreased responsiveness to stimulation of the alpha-2-adrenergic receptors.

Relaxations of isolated arteries evoked by a variety of vasodilators are caused indirectly by the release of EDRF from the endothelial cells (Furchgott and Zawadzki, 1980; De Mey and Vanhoutte, 1981; Furchgott, 1983). The present results confirm or demonstrate that both acetylcholine and ATP evoke endothelium-dependent relaxations in the aortic arch, the abdominal aorta, the brachiocephalic artery, and the pulmonary artery of the rabbit. Indeed,
the relaxations to acetylcholine are abolished and those to ATP are markedly diminished in these arteries in which the endothelium is mechanically removed. On the other hand, as was also reported by Furchgott and Zawadzki (1980), the relaxations to nitroglycerin are not dependent on the presence of endothelial cells in the rabbit arteries investigated.

In the introductory studies, we detected that the endothelium-dependent relaxations to acetylcholine and ATP were partially inhibited in three different arteries obtained from 8-week hypercholesterolemic rabbits. These positive results prompted us to study, in a more complete manner, the effect of hypercholesterolemia on endothelium-dependent and endothelium-independent relaxations in isolated rabbit arteries.

In the arteries of rabbits fed the 0.3% cholesterol-rich diet for 16 weeks, the relaxations to both acetylcholine and ATP were inhibited more than in the arteries obtained from rabbits fed the cholesterol-rich diet for 8 weeks. Since the thickness of the intimal lesions was more pronounced after 16 weeks than after 8 weeks of hypercholesterolemia, our results seem to indicate that the inhibition of the relaxations to both acetylcholine and ATP is dependent on the severity of the intimal lesions. It was indeed also evident that the endothelium-dependent relaxations were not inhibited to the same extent in the different arteries studied; thus, in the aortic arch, which—after 16 weeks of cholesterol feeding—was the most severely affected artery, acetylcholine no longer caused significant relaxations, whereas, in the abdominal aorta, the artery which was much less affected by the cholesterol-rich diet, the highest concentration of acetylcholine still evoked about 80% of the relaxation obtained in control tissues. Similarly, the reduction in ATP relaxation was more pronounced in the aortic arch than in the other arteries.

Moreover, our results also revealed that differences in the degree of lesion formation between different segments of a given artery (e.g., the aortic arch) were reflected in a more or less pronounced inhibition of the endothelium-dependent relaxation in this artery; e.g., two of seven aortic arches still showed significant relaxations to acetylcholine and these two aortas were damaged significantly less than the other five tissues (see Fig. 7). It thus was not unexpected to find a significant correlation between the degree of fatty streak formation and the inhibition of the endothelium-dependent relaxations as shown for acetylcholine in Figure 7.

In the more severely affected arteries (aortic arch and brachiocephalic artery), not only the endothelium-dependent relaxations to acetylcholine and ATP, but also the endothelium-independent relaxations to nitroglycerin were attenuated, compared to control responses. This finding may indicate that the cholesterol-rich diet has an additional effect on the ability of the vascular smooth muscle cells to relax. Such an effect can also explain why, in the aortic arches obtained from the 16-week hypercholesterolemic rabbits, the relaxations to ATP, which are due in part to a direct action on the smooth muscle cells (De Mey and Vanhoutte, 1981), are inhibited more than those obtained in deendothelialized aortic arches obtained from control rabbits. It should be pointed out here that, in the abdominal aorta, in which both relaxations to acetylcholine and ATP are significantly reduced after 16 weeks of hypercholesterolemia, the relaxations to nitroglycerin are not affected, and that even the most severely affected arteries (aortic arch) can still completely relax in response to higher concentrations of nitroglycerin. It should also be noted that in the case of acetylcholine, concentrations higher than $3 \times 10^{-6}$ M could no longer be used to relax the tissues, since they caused contractions due to activation of muscarinic receptors on the smooth muscle cells.

If the arterial lesions caused by the cholesterol-rich diet can inhibit specifically the endothelium-dependent relaxations in the isolated rabbit arteries, then it may be concluded that hypercholesterolemia, in one way or another, affects the release and/or the vascular activity of the EDRF. A decreased release of EDRF could be due to a loss or a malfunction of the vascular endothelial cells caused by the hypercholesterolemia. Histological examination of arterial segments obtained from both the 16-week control and hypercholesterolemic rabbits revealed that, at the stage of the disease where our experiments were performed, the arteries still contained the endothelial cells. Whether these endothelial cells can be classified as "normal," was beyond the scope of the present investigation. In hypercholesterolemic monkeys, fed a cholesterol-rich diet containing 0.5% cholesterol, endothelium denudation started to occur after 4 months of hypercholesterolemia; this denudation was observed in only the most severely affected arterial tissue: the iliac artery. However, already after 3 months of hypercholesterolemia, marked changes to the endothelium (irregularities, decreased density) became apparent (Faggiotto et al., 1984). These observations favor our interpretation that the endothelium was still present after 16 weeks of hypercholesterolemia. The question that needed to be answered then was: do the endothelial cells still release EDRF when they are stimulated with acetylcholine or ATP? Therefore, we investigated the release of EDRF in segments of abdominal aortas using a bioassay technique. The results obtained illustrate that acetylcholine can evoke similar amounts of EDRF release from both the control and the hypercholesterolemic abdominal aortas. At the concentrations of acetylcholine used to cause this EDRF release, segments of the abdominal aortas adjacent to the ones used for the bioassay studies showed a marked inhibition of the acetylcholine-induced relaxation (see Fig. 6). These observations, then, indicate that, in the hypercholesterolemic ar-
teries, vasodilators such as acetylcholine can still evoke release of EDRF from the endothelial cells; the inhibition of the relaxations caused by these vasodilators therefore must occur at a level subsequent to the EDRF release. One likely possibility is that thickening of the intimal layer of the arterial wall, due to infiltration and subendothelial accumulation of foam cells (forming fatty streaks and monocytes), and to infiltration of lipid-laden smooth muscle cells between the macrophages and the internal elastic lamella (Faggiotto et al., 1984), prevents the EDRF from reaching the vascular smooth muscle cells. The lesions will indeed alter the diffusion distances and the sequestration characteristics of the vascular segment. Other possibilities obviously are that steps subsequent to this "diffusion" of EDRF are involved. Thus, as shown by the decreased relaxation obtained with nitroglycerin, also, the mechanism leading to relaxation of the vascular smooth muscle cells may become impaired as the process of hypercholesterolemia continues.

Conclusions and Possible Implications of the Present Findings

In accordance with an earlier study using rabbit aorta (Henry and Yokoyama, 1980), our results indicate that during early stages of hypercholesterolemia in the rabbit, the contractile process of the vascular smooth muscle cells is not significantly altered. Our results also confirm that serotoninergic mechanisms may become supersensitive as the effects of the cholesterol-rich diet progress (see also Henry and Yokoyama, 1980; Yokoyama et al., 1983; Heistad et al., 1984). A major new finding is that the responsiveness to α-adrenergic stimuli appears to be decreased in hypercholesterolemic arteries. Our study, taken in conjunction with the findings reported by Henry and Yokoyama (1980) and Yokoyama et al. (1983), suggests that decreased α-adrenergic receptor-mediated contractility may become evident in blood vessels obtained from hypercholesterolemic rabbits.

The vascular smooth muscle processes leading to relaxation may become impaired as the hypercholesterolemic process progresses. This stage of decreased vascular smooth muscle relaxation is preceded by a stage of decreased endothelium-mediated relaxations. Our results indicate that a step, subsequent to release of EDRF (thickening of the intimal layer?) prevents the EDRF from evoking vascular smooth muscle relaxation. Dysfunction or loss of endothelium has been speculated to be of importance in atherosclerosis (Vanhoutte, 1983; Heistad et al., 1984). The finding discussed in our study—that endothelium-mediated relaxations are already impaired during early stages of hypercholesterolemia—certainly is in agreement with the proposed hypothesis.

It has been reported that, in patients with vascular diseases (coronary atherosclerosis, peripheral vascular disease), the incidence of vasospasm is increased (Schoeder et al., 1977; Robertson and Oates, 1978; Cipriano et al., 1979; Juergens et al., 1980; Miller et al., 1981; Waters et al., 1983; see also, Heistad et al., 1984). The fact that, in atherosclerotic blood vessels, the vasoconstrictor responses to serotonin are enhanced [as also described by Henry and Yokoyama (1980), Yokoyama et al. (1983), and Heistad et al. (1984)], while, at the same time, a process leading to relaxation is impaired (loss of endothelium-mediated relaxations), may play an important role in the pathogenesis of vasospasm.

Note added in Proof. The authors wish to state that at the time of submitting this manuscript for publication, they were aware that five abstracts (including two by the authors) had been published, which closely relate to the investigation reported above. All of these abstracts describe experiments showing that endothelium-mediated relaxations are inhibited in arteries obtained from hypercholesterolemic rabbits (Habib et al., 1984; Chappell et al., 1985; Coene et al., 1985; Verbeuren et al., 1985) and monkeys (Freiman et al., 1985).

We wish to thank M. Van Diest for his valuable help with the bioassay experiments; we also acknowledge the technical assistance of R. Van Hove and Y. Onsea, and are grateful to L. Van den Eynde for typing the manuscript. We are also indebted to Prof. L. Lepoutre (University Hospital of Antwerp) for determination of cholesterol and triglyceride levels and to Dr. H. Pöschner (Dr. Karl Thomae GMBH) for performing the histological examination of the thoracic aorta segments.

This study was supported by a grant of the FGWO and by a BEKALES award.

Dr. Coene's present address is: Department of Life Sciences, Janssen Pharmaceutica Research Laboratories, B-2340 Beerse, Belgium.

Address for reprints: Dr. Tony J. Verbeuren, Department of Medicine, UJA, Universiteitsplein 1, B2610 Wilrijk, Belgium.

References


Cohen RA, Shepherd JT, Vanhoutte PM (1983) Inhibitory role of
the endothelium in the response of isolated coronary arteries to platelets. Science 221: 273–274
Robertson D, Oakes JA (1978) Variant angina and Raynaud’s phenomenon. Lancet 1: 452

INDEX TERMS: Hypercholesterolemia • Rabbit arteries • Contraction • Relaxations • Endothelium-derived relaxant factor
Effect of hypercholesterolemia on vascular reactivity in the rabbit. I.
Endothelium-dependent and endothelium-independent contractions and relaxations in
isolated arteries of control and hypercholesterolemic rabbits.
T J Verbeuren, F H Jordaens, L L Zonnekeyn, C E Van Hove, M C Coene and A G Herman

Circ Res. 1986;58:552-564
doi: 10.1161/01.RES.58.4.552

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/58/4/552

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the
Editorial Office. Once the online version of the published article for which permission is being requested is
located, click Request Permissions in the middle column of the Web page under Services. Further information
about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/